Journal of
DAIRY SCIENCE

Vol. 44 May, 1961 Number 5

CONTENTS

PEOPLE AND EVENTS 5

DAIRY TECHNOLOGY SOCIETIES 16

GENERAL INTEREST:

Antibiotics in Milk—A Review.
J. L. Albright, S. L. Tuckey, and G. T. Woods 779

Awards of A.D.S.A.—A Review.
G. M. Trout 955

Research and Education Are the Keys to Agricultural Progress.
R. E. Hodgson 964

A.D.S.A. and Industry Relationships.
H. F. Judkins 968

Call for Papers for Eastern Meeting of A.D.S.A.
W. F. Shipe 981

RESEARCH PAPERS:

Relation of Milk Serum Proteins and Milk Salts to the Effects of Heat Treatment on Rennet Clotting.
A. Kannan and Robert Jenness 808

Apparent Location of Lipase in Casein.
J. D. Skean and W. W. Overcast 823

Characteristics of Commercial Nonfat Dry Milk for Cottage Cheese.
H. E. Randolph and T. Kristoffersen 833

Effect of Peptide-Rich Extracts on Acid Production by Oxytetracycline and Penicillin-Resistant Starters.
H. E. Kennedy 844

Comparison of Flavor and Some Chemical Properties of Instantized and Noninstantized Nonfat Dry Milk.
J. B. Mann and J. M. McIntire 851

Accuracy of the Plastic Bead Method of Measuring Solids-not-fat of Milk Under Field Conditions.
R. C. Lamb, L. D. McGilliard, and J. R. Brunner 856

Further Comparisons of Alfalfa Hay and Alfalfa Silage for Growing Dairy Heifers.
J. W. Thomas, L. A. Moore, and J. F. Sykes 862

(Continued on inside front cover)

CONTENTS

(Continued from front cover)

Nutritive Value of Reed Canarygrass as Hay when Grown with Various Nitrogen Levels.
W. V. Chalupa, J. L. Cason, and B. R. Baumgardt 874

Livability of Purebred vs. Crossbred Dairy Cattle.
F. N. Dickinson and R. W. Touchberry 879

Progestins in Corpora Lutea, Ovaries, and Adrenals After Estrus and Breeding of Normal and Abnormal Cows.
R. E. Erb and F. Stormshak 888

Variation in Estrual Cycles of Holstein-Friesian Cattle.
S. E. Mares, A. C. Menge, W. J. Tyler, and L. E. Casida . . . 897

Effect of Total Digestible Nutrient Intake from Birth to Four Years of Age on Growth and Reproductive Development and Performance of Dairy Bulls.
R. J. Flipse and J. O. Almquist 905

Electron Microscopic Study of Mature Bovine Spermatozoa.
Donald F. Rahlmann 915

Part Lactations. III. Progeny Testing with Part Lactation Records.
S. R. Searle 921

Adrenalectomy of the Calf and Its Effects on Various Blood Constituents.
V. L. Estergreen, Jr. and N. L. VanDemark 928

Factors Affecting the Secretion of Iodine^{131} into Milk of Lactating Goats.
E. P. Reineke 937

TECHNICAL NOTES:

Lipolysis by a B-Esterase Preparation from Milk.
R. G. Jensen, G. W. Gander, J. Sampugna, and T. L. Forster . . 943

Interesterified Butter Oil.
Herman D. Weihe 944

Milking Efficiency and Durability of Milking Machine Inflations of Various Compoundings.
Gerald M. Ward, Virgil R. Elsey, and Herbert Jordan 947

OUR INDUSTRY TODAY:

A Comparison of Scores and Measurements of the Udder Proportions.
Ivar Johansson and E. E. Ormiston 950

Container Problems in Refrigerated Warehouses.
W. A. Maclinn 952

ASSOCIATION AFFAIRS 955

Abstracts of Papers Presented at Southern Meeting of A.D.S.A. . . . 969

BOOK REVIEWS 982

JOURNAL OF DAIRY SCIENCE

OFFICIAL PUBLICATION OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

E. O. HERREID, Editor-in-Chief, University of Illinois
Editorial Address: Station A, Box 718, Champaign, Illinois
Phone: EMpire 7-7073

EDITORIAL BOARD

C. K. JOHNS, Ottawa, Canada
H. D. EATON, Storrs, Conn.
R. E. ELY, Reno, Nevada
F. A. KUMMEROW, Urbana, Ill.
N. L. JACOBSON, Ames, Iowa
ROBERT JENNESS, St. Paul, Minn.
J. J. JEZESKI, St. Paul, Minn.
J. E. LEGATES, Raleigh, N. C.
J. P. MIXNER, Sussex, N. J.
DURWARD OLDS, Lexington, Ky.
STUART PATTON, University Park, Pa.
M. L. SPECK, Raleigh, N. C.
E. L. JACK, Davis, Calif.
N. L. VANDEMARK, Urbana, Ill.
EVERT WALLENFELDT, Madison, Wis.
G. M. WARD, Manhattan, Kan.
U. S. ASHWORTH, Pullman, Wash.
R. W. TOUCHBERRY, Urbana, Ill.

COMMITTEE ON JOURNAL MANAGEMENT

F. J. DOAN, Chairman
Department of Dairy Science, Pennsylvania State University, University Park, Pa.

J. F. CAVANAUGH
H. A. HERMAN
E. O. HERREID, ex officio
H. F. JUDKINS, ex officio

OFFICERS OF THE ASSOCIATION

R. E. HODGSON, President
Animal Husbandry Research Branch, USDA
Beltsville, Md.

E. L. JACK, Vice-President
Department of Food Science and Technology,
University of California, Davis, Calif.

H. F. JUDKINS. Sec.-Treas.
32 Ridgeway Circle, White Plains, N. Y.

E. O. HERREID, Journal Editor-in-Chief
Champaign, Ill.

DIRECTORS

S. T. COULTER, St. Paul, Minn.
F. J. DOAN, University Park, Pa.
A. C. FAY, North Miami, Fla.
N. P. RALSTON, East Lansing, Mich.
J. T. REID, Ithaca, N. Y.
E. C. SCHEIDENHELM, Lexington, Ky.
G. H. WISE, Raleigh, N. C.
E. O. HERREID, ex officio
H. F. JUDKINS, ex officio

ADMINISTRATIVE INFORMATION

Manuscripts

Manuscripts for publication in the Journal of Dairy Science should be submitted to the editor, E. O. Herreid, at the address above. See January, 1954, issue, page 103, for information concerning the preparation of manuscripts, or send for reprint, "Information for Authors."

Advertising

Advertising plates, copy, insertion orders, contracts, and requests for information relating to advertising in the Journal should be addressed to the Journal of Dairy Science in care of The Garrard Press, 510–522 North Hickory Street, Champaign, Illinois.

Nonmember Subscriptions

Nonmember subscriptions are $15.00 per volume in the United States and Canada; $15.50 in all other countries, net and postpaid. New subscriptions and renewals begin with the first issue of the current volume. Renewals should be sent in by December 1 of the current year for the following calendar year to avoid a break in the series. Subscriptions should be sent to the Journal of Dairy Science in care of The Garrard Press, 510–522 North Hickory Street, Champaign, Illinois.

Subscriptions for the British Isles and the British Empire should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Street, Covent Garden, London, W.C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert Street, St. Leonards, N.S.W., Australia.

Changes of mailing addresses, post office notices of undeliverable copies, inquiries about copies lost in the mail, and all other matters pertaining to the mailing list for nonmember subscribers should be sent to The Journal of Dairy Science in care of The Garrard Press at the above address. Claims for copies of the Journal of Dairy Science lost in the mails must be received within 30 days (90 days foreign) of the date of issue. Notice of change of address must be received two weeks before date of issue.

Memberships

Membership dues in the Association are $10.00 a year and should be sent to H. F. Judkins, Secretary-Treasurer, 32 Ridgeway Circle, White Plains, New York. Renewals should be made not later than December 1 each year.

Changes of mailing addresses, inquiries about copies lost in the mail, requests for back copies and all other matters pertaining to the mailing list for members of the American Dairy Science Association should be sent to Mr. Judkins at the above address. Claims for copies of the Journal of Dairy Science lost in the mails must be received within 30 days (90 days foreign) of the date of issue. Notice of change of address must be received two weeks before date of issue.

Form 3579 to be returned to Garrard Press

Printed by The Garrard Press, 510–522 North Hickory Street, Champaign, Illinois. Entered as second-class matter at the post office at Champaign, Illinois, under the act of March 3, 1879.

Copyright, 1961, by the American Dairy Science Association. Printed in the U. S. A.

Augustus H. Fiske, Jr.
President
FISKE ASSOCIATES, INC.

From the President:

IMPORTANT MESSAGE on FISKE CRYOSCOPES

Unauthorized use of the FISKE name and misleading statements regarding FISKE MILK CRYOSCOPES have come to my attention. These are the facts and here is a statement of our policy:

There is not now, and never has been, a "New 1961 Advanced Fiske Milk Cryoscope" on the market. We are the developer and manufacturer of the FISKE MILK CRYOSCOPE—the instrument around which the official procedures, as described in STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS, 11th Edition, 1960, APHA, and in LABORATORY MANUAL, Milk Industry Foundation, 3rd Edition, 1959, are written. The Fiske Cryoscope meets the specifications of the A.O.A.C.

Fiske Associates has prepared an accurate and informative booklet on Practical Precision Cryoscopy which is available free of charge.

VARYING SCALES

Users of Fiske Cryoscopes have been approached with proposals to change certain scale markings and make other modifications on their instruments, purporting to simplify their use.

Fiske Associates does not sanction these changes because they limit the flexibility and accuracy of the instrument, which are important to maintain in the rapidly developing art of Milk Cryoscopy.

All advancements in the FISKE method of Milk Cryoscopy—the development of the salt "equivalent sucrose" standards, vibrating rod stirring, and simultaneous "buzz" freezing—have originated in our laboratory. Our latest development is "shock" freezing. This feature, an important contribution to the field of precision Cryoscopy, is available only in FISKE instruments.

The first Fiske Cryoscope was built nearly a decade ago. It proved highly successful in operation and for the first time in history made precision cryoscopy a routine laboratory procedure. The first Fiske Milk Cryoscope was introduced to the dairy industry in 1955. Several hundred Fiske Milk Cryoscopes have been built since then and are used in the laboratories of leading milk producers, processors, testing laboratories, and major research institutions throughout the world.

Address any inquiries directly to me at the plant, or through our authorized agents. We will be pleased to reply promptly.

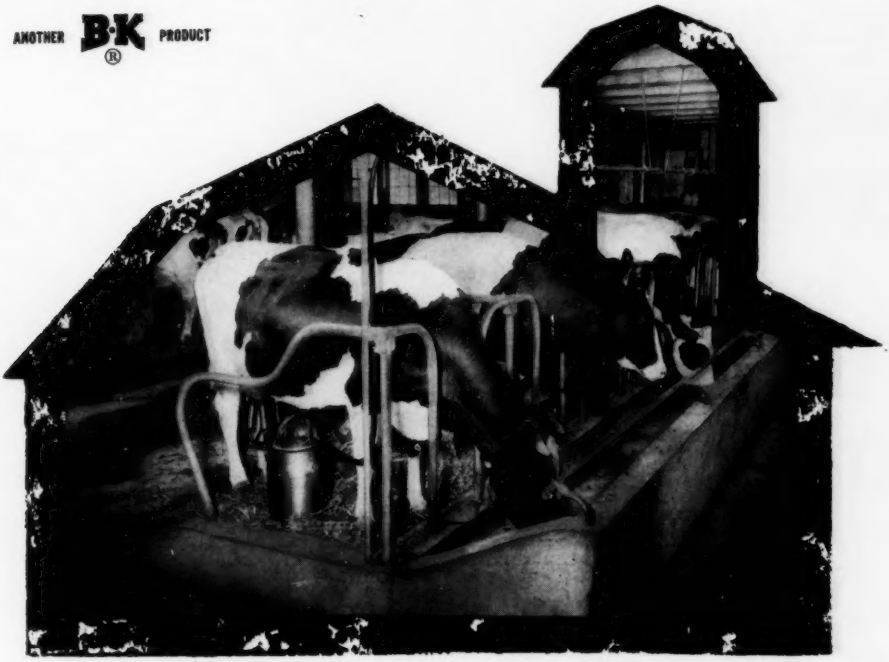
Augustus H. Fiske Jr.

Fiske

FISKE ASSOCIATES, INC., BETHEL, CONNECTICUT, PIONEER 8-8369

Creators of Precision Electronic Devices

ANOTHER B-K® PRODUCT



IN THE LAB PROVED ON THE FARM

Pennsan®

WORKS MORE WAYS TO INCREASE MILK QUALITY AND REDUCE SANITIZING COSTS

Here's a new concept that offers *more* advantages in sanitizing and cleaning: PENNSAN . . . a unique chemical sanitizer. It's the superior bactericide to serve the needs of modern dairy sanitizing.

PENNSAN's outstanding properties have been established by extensive lab tests and highly successful dairy farm use. (Test results on request.) These all demonstrate that PENNSAN:

- **Removes and prevents milkstone and films**
- **Works effectively even in hardest water**
- **Does not corrode stainless steel**
- **Controls bacteriophages—does not affect starter cultures**
- **Retains bactericidal effectiveness longer**
- **Sanitizes and cleans *more* equipment**

Write now for free booklet to B-K Dept.
PENNSALT CHEMICALS CORPORATION
East: 3 Penn Center, Phila. 2, Pa.
West: 2700 S. Eastern Ave., Los Angeles, Cal.

Pennsalt® Chemicals
ESTABLISHED 1850

For Consistent QUALITY... TEXTURE... and FLAVOR THAT SELLS! use...

OK BRAND

CORN SYRUPS

For those desiring an economical source of body-building solids and a smooth, "chewy" body in the ice cream, we recommend our OK REGULAR corn syrup. Plants which desire more sweetness in the syrup with a little less emphasis on "chewiness" or body of the ice cream, use Hubinger's OK HIGH DEXTROSE. Those ice cream operators who manufacture a product with a very low overrun and a fair high serum solids, use Hubinger's HI-SWEET syrup in their mixes. HI-SWEET is an enzyme-converted syrup with a high dextrose equivalent resulting in at least 65% sweetness value and a clean, neutral flavor. One batch will convince you. Try OK Corn Syrups, soon!

Whatever your ingredient problems may be, we'd like to help you solve them. "The Man From Hubinger" will welcome the opportunity to show you the advantages of using OK BRAND Syrups in your formulas. Call today for prompt service.

THE HUBINGER COMPANY
Keokuk, Iowa
NEW YORK • CHICAGO • LOS ANGELES • BOSTON • CHARLOTTE • PHILADELPHIA

PEOPLE AND EVENTS

Dr. L. A. Black Honored

L. A. BLACK, Chief of Milk Sanitation at the U. S. Public Health Service's Robert A. Taft Sanitary Engineering Center, 4676 Columbia Parkway, Cincinnati, Ohio, received an Honor Award from the U. S. Department of Health, Education, and Welfare, Tuesday, April 11, in Washington, D. C. Dr. Black is a member of the Milk and Food Research Program at the Sanitary Engineering Center.

He received the award for his work in the development and unification of laboratory methods and standards in milk and food sanitation. He is recognized as an authority in milk bacteriology. Research conducted by him includes collaborative studies in the development and standardization of laboratory methods for the examination of milk, studies leading to the development of methods for testing bactericides, and extensive studies for evaluating the performance of laboratories. Dr. Black's contributions to the scientific literature include more than 80 articles.

Dr. Black is also a member of the Society of American Bacteriologists, the American Dairy Science Association, and is a fellow of the American Public Health Association. He is now Chairman of American Public Health Association Subcommittee on Standard Methods for the Examination of Dairy Products, Chairman of the Laboratory Committee of the National Conference for Interstate Milk Shipments, and is a member of the Public Health Committee of the American Dairy Science Association.

Late in 1960, Dr. Black received the Annual Citation Award of the International Association of Milk and Food Sanitarians. This award, given annually to the individual who has given outstanding service to the Association, and who has advanced the cause of sanitation and the sanitarian, also was in recognition of his work in milk bacteriology. In the same year he also received a cash award for superior performance in his work at tne Sanitary Engineering Center.

Dr. Black has been associated with the Public Health Service since 1941. He and his wife live at 3722 Pleasant Street, Mariemont, Ohio.

R. I. Meyer Appointed to Food and Drug Administration

R. I. MEYER, for 12 yr. with the Quartermaster Food and Container Institute for the Armed Forces, has accepted a position with the Food and Drug Administration in Washington, D. C. He will be in the Food Research Branch, working under the direction of DR. WILLIAM HORWITZ, and will be the specialist for edible oil-containing foods.

R. I. Meyer

Since 1958, Mr. Meyer has been Chief of the Dairy, Oil and Fat Products Branch at the Quartermaster Depot of the Armed Forces in Chicago. In 1956 he received Honorable Mention from the Quartermaster Corps for his work on canned cheese products for the Armed Forces.

Mr. Meyer earned the M.S. degree in Dairy Bacteriology at the University of Illinois in 1949.

UNIVERSITY NEWS

Connecticut

Dairy Day and Annual Dairy Club Dinner at Connecticut

A very successful Dairy Manufacturing Day was held at the University of Connecticut on March 22, 1961. The program dealt with timely information and current problems in the dairy industry. Topics and speakers included: Greetings by DEAN W. B. YOUNG of the College of Agriculture; Two Years Under the Federal Order—and a Look Ahead, DR. S. JOHNSON, Department of Agricultural Economics, University of Connecticut; Dating of Milk, G. HELMBOLDT, Production Manager of Metropolitan Sealtest Foods; Operating with Smaller Margins, DR. R. D. APLIN, agricultural economist at Cornell University; Researches in Advanced Age, DR. J. F. CUMMINS, Chief of the Geriatric Clinic, Veterans Administration, Boston, Massachusetts; Fats, Fads, and the Diet, R. W. SHAFER, Eastern Regional Manager of the National Dairy Council; Problems Facing the Dairy Industry, DR. W. A. COWAN, Head of the Department of Animal Industries, University of Connecticut; and Research Highlights at the University of Connecticut by DR. R. G. JENSEN.

Dairy Farmer's Day was held concurrently

Projects, Consultation, and Production Control Services in Biochemistry, Chemistry, Bacteriology, Toxicology—Insecticide Testing and Screening.

Write for price schedule.

WISCONSIN ALUMNI RESEARCH FOUNDATION

P.O. Box 2217-X, Madison 1, Wisconsin

New Holland Compact Hayliner 65, with Bale-Thrower, costs no more than many balers *without* automatic wagon-loading feature!

Now bale twice as fast with half the labor!

Today there's a new way to bale—that lets *one* man bale *and load* faster than a 2- or 3-man team! Fully proved on hundreds of farms. Choice of five baler models, including new Compact model. New compact model with automatic wagon-loading costs no more than many balers alone!

Here's the biggest farming news of '61—*proved, practical one-man haying!* Now, one man can bale *and load* automatically—in half the time it takes with manual loading!

How it works

The new system consists of a New Holland Hayliner—the baler that outsells every other—plus a simple, sure-fire mechanism that "airlifts" the bales into the trailing wagon. You position the bales right from the tractor seat. Doesn't miss—even on turns.

What it costs

Because of its simplicity, this New Holland baling method costs surprisingly little. In fact, the new Compact Hayliner 65 with Bale-Thrower costs no more than many balers without automatic wagon loading!

Any Hayliner will soon pay for itself:

- **You will save your own time,** because you will bale steadily at much faster speed.
- **You will save labor costs.** Instead of loading wagons your helpers can be doing other, more profitable work.

If you already own a twine-tie New Holland Hayliner, you can easily convert to one-man baling and loading. If your present baler is another make, your New Holland dealer will offer you a generous trade-in, pay-as-you-save terms.

FREE BOOKLET! Write New Holland Machine Company, Division of Sperry Rand Corp., New Holland, Pa.

NEW HOLLAND
"First in Grassland Farming"

this year to enable people to hear talks in both sections. The group was welcomed by DR. W. A. COWAN, Head of the Animal Industries Department. Other speakers on the morning program were DR. RUFUS MUNSELL, Extension Agronomist, and C. B. BENDER, director of grassland farming research for Sperry Rand Corporation of New York.

E. S. HARRISON, manager of Harden Farms of Camden, New York, discussed Making the Best Use of Roughage during the afternoon session. Available control facilities for diseased dairy cattle were discussed by DR. CHARLES HELMBOLDT, head of the Department of Animal Diseases at the University of Connecticut.

In the evening the Annual Dairy Club Dinner was held, and an outstanding person from the field of Dairy Manufacturing and one from Dairy Production were honored. This year the student organization honored LEWELLYN DIBBLE, Production Manager for Bryant and Chapman's Hartford Sealtest plant. Mr. Dibble graduated from the University of Connecticut in 1927. He has been with the same company since September, 1927, working up through the laboratory, research, Sealtest supervision to his present position.

GERALD HAYS of North Granby was the dairy farmer honored this year at the banquet. He has been active in town affairs, the Grange, C.M.P.A., Eastern States Farmers Exchange, and the artificial breeding associations. He has held important offices in these organizations and is president of the New England Selective Breeding Association.

Nebraska

Approximately 60 Nebraska dairy plant operators participated in a Market Milk Conference at the University of Nebraska April 4 and 5. The conference was sponsored by the Department of Dairy Husbandry and the Agricultural Extension Service.

Subjects discussed included Education for Agreiultural Industry, Quality and Quality Tests, Possibilities for Diversification in the Milk Plant, Effect of Processing Procedures on Fluid Milk Products, Chocolate Flavored Milk Problems, Automation in the Dairy Industry, Off-Flavors in Bulk Milk, Losses from Your Milk Filler, Cultures and Starters, and Factors Affecting Shelf Life of Cottage Cheese. In addition to the lectures, demonstrations of the long and short methods of making cottage cheese were presented.

Speakers included DR. PHIL KEENEY, Pennsylvania State University; DR. W. D. SIMONET, Robert A. Johnston Co.; R. C. HOPKINSON, Cherry-Burrell; R. L. ZIEGLER, Ziegler & Son; M. W. HALES, Klenzade; and DR. F. E. ELDRIDGE, DR. KHEM SHAHANI, DR. R. B. MAXCY, and K. M. NILSON of the University of Nebraska.

THROUGH KLENZADE SANITATION SURVEYS

Put Today's Best Methods

TO WORK FOR YOU

The economics of dairy processing are forcing a sharper look at cost reduction and quality control. Klenzade is in tune with this forward trend . . . ready to help you . . . in all departments. Somewhere within the range of manual cleaning . . . CIP cleaning . . . semi-automatic . . . or fully automated cleaning . . . there is an ideal set-up for you. A Klenzade Sanitation Survey will reveal it — what to do, how to do it. And a Klenzade Program will place in your hand positive daily control of clean-up costs and quality . . . so far reaching and efficient that it will even include the trucks in your garage. Write for details.

YES I'M INTERESTED IN A SANITATION SURVEY for QUALITY & COST CONTROL

INDIVIDUAL .....

POSITION .....

COMPANY .....

ADDRESS .....

Mail to: KLENZADE PRODUCTS, INC.
Systematized Sanitation All Over the Nation
DEPT. 20E BELOIT, WIS.

FIGHT MASTITIS WITH A MILKER?

Yes! Gentle De Laval milking can help you do it!

It's no secret that rough milking machines, or improper milking, can predispose dairy cows to mastitis. That's why you should know there's a method of milking that's remarkably *gentle* on cows' sensitive teats and udder tissues . . . so gentle it can help you and dairymen in the fight to keep herds mastitis-free.

De Laval milking provides maximum protection to udders to help any mastitis prevention program work better. Here are the reasons why:

1. Other milkers use wide-bore liners that cause cows' teats to "balloon" to fill extra space. De Laval uses narrow-bores that fit snug, reduce impact when liners collapse, minimizing danger of udder injury.

2. Our magnetic pulsation assures *absolutely uniform milking* with never a variation. Pulsators are factory set, won't get out of adjustment.

3. De Laval cuts vacuum drop-offs that cause slow, erratic milking. Our *rotary* vacuum pump assures rapid vacuum recovery for faster, more uniform milking.

These exclusive features are the difference between harsh milking and the *gentle* milking dairymen need for maximum protection of cows' udders to help prevent mastitis. Gentle milking is built right into every De Laval milker . . . Suspended, Pail-Type, Cow-to-Can® and Combine Milker®. For further information, write: The De Laval Separator Co., Poughkeepsie, N. Y., or 5724 N. Pulaski, Chicago 46, Ill., or 201 E. Millbrae Ave., Millbrae, California.

pioneers in better milking for more than 75 years

DE LAVAL

New Hampshire

DR. H. A. KEENER has been appointed Dean of the University of New Hampshire's College of Agriculture. He will assume the post July 1. Dr. Keener will succeed DEAN H. C. GRINNELL, who is retiring this year because of the mandatory age retirement for University administrators.

Dr. Keener, who is Professor of Dairy Science at the University and Director of the Agricultural Experiment Station, will continue temporarily in the latter position. He has been a member of the University faculty for 20 yr., serving first as Instructor in Animal and Dairy Husbandry and later as Assistant Professor, Associate Professor, and Professor of Dairy Husbandry.

The newly appointed Dean has been prominent for many years in the field of animal nutrition research and has written more than 30 scientific papers. He is the author, with Henry F. Judkins, of the book, Milk Production and Processing, published in 1960, and has served on the University's Research Council.

Dr. Keener is a native of Pennsylvania and holds the B.S. and Ph.D. degrees from Pennsylvania State University and a M.S. degree from West Virginia University. He is a member of Sigma Xi, national honorary research society, Phi Kappa Phi, honorary scholastic society, the American Dairy Science Association, the New York Academy of Sciences, and the American Society of Animal Production.

New York

Thirteen Nutrition Fellowships Established by Food Industry at Columbia University

A critical shortage of trained nutritionists and food technologists in the underdeveloped countries is being solved through a $78,000 grant by seven major food processors in the United States. The grant will bring 13 foreign students to study at Columbia University's Institute of Nutrition Sciences.

Students specially qualified and selected for the project will be enrolled in Columbia's recently established Institute of Nutrition Sciences under a system of fellowships set up by the seven firms. The program is designed to train leaders who, on return to their homelands, can train others and develop effective public health and nutrition programs in the underdeveloped areas, with particular emphasis on those countries in which malnutrition is a significant problem.

Companies agreeing to co-sponsor and underwrite the program are: The Borden Company, three fellowships; Campbell Soup Company, one fellowship; Corn Products Company, three fellowships; General Foods Corporation, one fellowship; General Mills, Inc., one fellowship; H. J. Heinz Company, three fellowships; The Pillsbury Company, one fellowship. Each of the fellowships will be identified by the name of the sponsoring company.

The program is designed to give advanced students an understanding of food supplies, food technology, human nutritional requirements, the nature of deficiency diseases, nutrition in preventive medicine, and the problems involved in changing the food habits of large groups of people.

Oregon

Plans Announced for Second Foods Symposium

Oregon State University has announced plans for a second biennial Symposium on Foods September 11, 12, and 13. Theme for the conference will be Oxidative Deterioration of Food Lipids, a major problem in the manufacture and storage of dairy and food products.

The symposium, arranged by the University's Department of Dairy and Food Technology, will bring together leading United States and foreign authorities for discussions on the undesirable chemical reactions occurring in man's food supply and their implications in the human diet. Sixteen authorities on lipid oxidation will speak during the three-day conference, with discussion and question periods planned after each lecture. Two scientists from abroad will be on the program along with the leading United States experts. They will be DR. C. H. LEA, University of Cambridge, England, and DR. NOBORU MATSUO, Tokyo Medical College, Tokyo, Japan.

Ontario

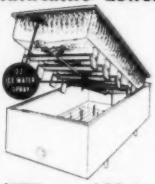
A Dairy Plant Fieldman's Conference was held at the Ontario Agricultural College March 23 and 24. This was the first fieldman's conference held at O.A.C. PROF. I. E. PARKIN of The Pennsylvania State University presented two papers, Growth, Expansion, and Mechanization of the Dairy Enterprise and Mastitis Prevention. Other speakers included J. DOAN of The Carnation Company and T. DICKENSON, Chief Field Supervisor, The Borden Company. O.A.C. staff members taking part in the program included PROF. F. W. HAMILTON, PROF. A. LEGGATT, and DR. D. R. ARNOTT. The success of the program indicates that it will become an annual affair.

An ice cream short course was conducted at the Ontario Agricultural College, April 3-7. Nineteen students from five Canadian Provinces and two foreign countries attended. Two Danish students, H. LARSEN and JENS PEDER-

NO FROZEN MILK IN A

DARI-KOOL
BULK MILK COOLER

● **Cools Milk Faster and Maintains Lower Blend Temperatures Than Any Other Cooler**



33° ice-water cooling removes milk heat fast. Does not lower butterfat test by freezing milk.

● **Cools Milk for Less Than 1KW per 100 lbs. on a Yearly Average — and Under Full Load**

Metered tests run at Young's Dairy, Twin Falls, Idaho, Dari-Kool used only .83 KW per 100 lbs. Send for this test.

● **Protects Milk from Spoilage During Electric Power or Compressor Failures —**

Dari-Kool's large reserve ice-bank protects milk from spoilage during such emergencies.

* In a national farm magazine preference survey of tank owners (all brands) Dari-Kool is now the 3 to 1 favorite over the second brand. Send for copy of survey.

DAIRY EQUIPMENT CO., Madison, Wis.

SEN, utilized modern jet service to attend this 1-wk. course. The course covered all phases of mix formulation, mix preparation, and freezer operation.

A Cottage cheese workshop was conducted at the Ontario Agricultural College on February 28, March 1 and 2. There were 61 cheesemakers in attendance. DR. D. M. IRVINE outlined the care of cultures and the mechanical sequences in Cottage cheese manufacture.

The 1961 Dairy Short Course has been completed at the Ontario Agricultural College. Thirty-one students from three provinces attended the course, which covered fundamental and practical procedures in manufacturing and testing of dairy products, dairy chemistry, dairy plant management, and dairy bacteriology.

Saskatchewan

D. L. Gibson to India

The University of Saskatchewan has granted DR. D. L. GIBSON, Head of the Department of Dairy Science, a year's leave of absence to proceed to India with the Food and Agriculture Organization of the United Nations. Some time will be spent in New York, London, and Rome.

On arrival in India, Dr. Gibson will spend three months lecturing at the Karnal Dairy Research Institute, north of New Delhi, and will continue to pursue research in the field of ultra-high temperature heat treatment of dairy products. The remainder of the time will be spent at the Dairy Research Institute in Bangalore, where he will act as a research consultant in an advisory capacity.

Mrs. Gibson and daughters, Mary Jane and Ellen Anne, will accompany him.

His address in India up to August 1, 1961 will be: F.A.O. Dairy Specialist, c/o Dairy Research Institute, Karnal, Punjab, India. After August 1, 1961: F.A.O. Dairy Specialist, c/o Dairy Research Institute, Bangalore, Mysore, India.

INDUSTRY NEWS

Ralston Purina Research Fellowships Award

The three Ralston Purina Fellowships this year were won by: H. H. HODSON of Ames, Iowa, and a graduate of Iowa State University, where he will also do his graduate work; A. J. LEE, of Quebec, Canada, and a graduate of Macdonald College. He has selected Cornell University for his graduate work. J. M. PHIL-

HOW KRAFT SERVES THE DAIRY INDUSTRY WITH STABILIZERS AND EMULSIFIERS

Kraft offers the dairy industry a wide variety of these ingredients for manufacturing ice cream, sherbets and frozen novelties. Each Kraft stabilizer and emulsifier is produced to meet Kraft's high standards and is designed to fill a specific dairy need. Accordingly, each helps insure that the dairy's end product will be of uniform goodness.

STABILIZERS FOR ICE CREAM

KRAGELEEN ICE CREAM STABILIZER. Used in any ice cream mix for vat pasteurization or HTST where the mix is preheated. Made from Kraft's own specially processed Irish moss and designed to properly stabilize the mix and give it good body and a creamy melt-down.

KRAGELEEN FL. A special formula, developed specifically for HTST, to produce a top-quality ice cream.

STABILIZER FOR SHERBETS

SHER-STAY SHERBET STABILIZER. A new Kraft development for stabilizing sherbet, Sher-Stay reduces spurting and improves texture and body.

STABILIZER FOR FROZEN NOVELTIES

KRABYN STABILIZER. In new instant form . . . an excellent stabilizer for frozen novelties, designed to produce a smooth texture, proper viscosity and prevent the growth of ice crystals.

EMULSIFIER FOR ICE CREAM

KRAFT'S ICE CREAM EMULSIFIER. Kraft Emulsifier for ice cream and frozen confections. The 100% emulsifier that gives whipability to ice cream without interfering with efficient freezing. It improves texture and makes a dry ice cream.

EMULSIFIER-STABILIZERS FOR ICE CREAM

KRAFT EMULSIFIER-STABILIZER. This combination product functions as both a stabilizer and an emulsifier and offers the advantage of handling only one ingredient. It allows freezing the mix immediately after processing and gives better control of over-run.

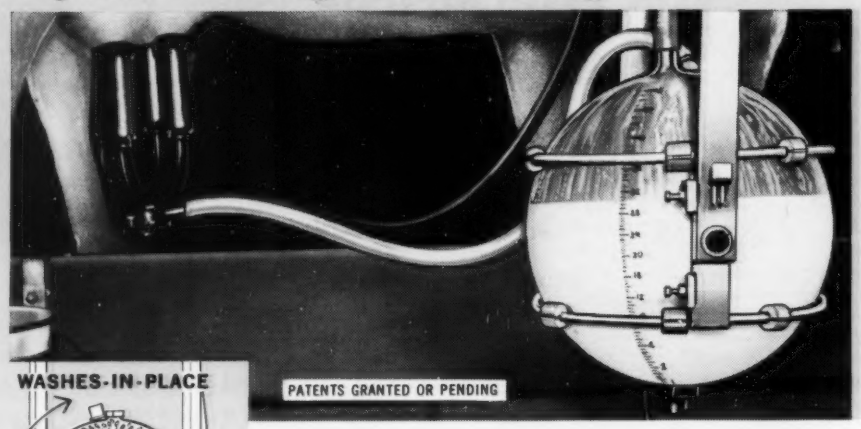
KRAFT 17 ES. Combining the advantages of both a stabilizer and an emulsifier, this ingredient produces a dry ice cream, controls over-run and is uniform and economical.

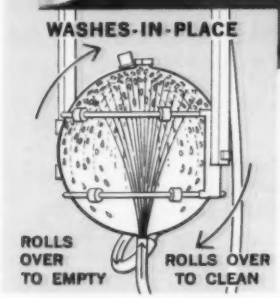
KRAFT

To obtain free samples for classroom work, ask your Kraft Man or write
KRAFT FOODS DAIRY SERVICE DIVISION
CHICAGO • NEW YORK • GARLAND, TEXAS • SAN FRANCISCO

New CHORE-BOY *Roll-O-Measure*

The Only Practical Means of Determining Individual Milk Production Per Cow—with Pipeline Milking and Cleaning-In-Place!





Note to Breed and D.H.I.A. Officials

Chore-Boy has incorporated in the new Roll-O-Measure features suggested by Breed and D.H.I.A. officials, for greater accuracy of measuring and obtaining butterfat samples. We will be pleased to loan government or state officials Roll-O-Measure Units, on which they may run their own tests, to determine if official approval of this method for breed and D.H.I.A. testing should be given. The company now guarantees laboratory precision and accuracy of calibrations within 2% of actual weight.

The calibrated glass Chore-Boy Roll-O-Measure shows exactly how much milk each cow produces at every milking. It enables the operator to spot the poor producers and keeps an accurate check on all the cows. Bloody milk and milk from fresh cows can be withheld from the milkline. The milk is not elevated while the cow is being milked. The large vacuum reserve close to the udder maintains a steady vacuum. The low-level flow of milk is easy, short and fast. The operator can easily see when milk flow stops. These advantages combined with Chore-Boy's gentle low-vacuum milking helps eliminate one of the major causes of mastitis. The Roll-O-Measure sphere is the ideal shape for visibility, compactness and cleaning-in-place. There are no corners, cracks or crevices. Send today for complete information on Chore-Boy Milking equipment.

CHORE-BOY
LOW-VACUUM
MILKING SYSTEMS
© ®

CHORE-BOY MANUFACTURING CO., Cambridge City, Ind.

POT of Stillwater, Oklahoma, and a graduate of Oklahoma State University, where he will also do his graduate work.

DR. N. L. JACOBSON of Iowa State University represented the American Dairy Science Association on the Awards Selection Committee.

Beatrice Foods Organizes Plant in Malaya

H. H. TENGKU ABDUL RAHMAN, Prime Minister of the Federation of Malaya, officially opened the new Beatrice Foods (Malaya) Ltd., dairy plant in Petaling Jaya, Malaya, in official ceremonies on February 7.

The dairy operation, initially producing canned sweetened condensed milk, was organized jointly by Beatrice Foods Company, and Fraser & Neave, Ltd., of Singapore, a publicly owned Malayan firm. It is the first American plant to be built in Malaya under that nation's new pioneer economic development program. Petaling Jaya is a suburb of the Malayan capital, Kuala Lumpur.

Others taking part in the ceremonies, in addition to the Prime Minister, included H. M. BYINGTON, JR., U. S. Ambassador to Malaya; COL. C. M. BENNETT, New Zealand High Commissioner to Malaya; DATO ONG YOKE LIN, Malayan Minister of Health; the Imam, Moslem religious advisor to the Prime Minister;

Beatrice Foods Plant in Malaya.

MAX LEWIS, board chairman of Beatrice Foods (Malaya) and a managing director of Fraser & Neave; TAN CHIN TUAN, board chairman of Fraser & Neave. Also participating were: J. F. HAZELTON, executive vice-president of Beatrice Foods Co., B. W. CANNON, Western regional vice-president of Beatrice Foods Co.; A. G. MARCUS, former Hawaiian district manager for Beatrice Foods Co. and now general manager for the Beatrice South East Asia operations; DR. G. W. SHADWICK, general director of quality control and foreign service for Beatrice Foods Co., and K. C. LEONG, general manager of the Beatrice Foods (Malaya) plant.

Construction of the plant began last spring and was completed in early November. Special designed machinery valued at $170,000 was shipped from Chicago to be installed in the plant, after 3 mo. of testing under actual operating conditions in the Beatrice Foods Co. plant (1526 S. State St.), Chicago. Kenneth Barker, a Beatrice Foods employee for 3 yr., and experienced in the dairy industry, is production manager of the Malayan plant.

Stainless steel equipment, tiled floors and walls, and ultra-modern decor make the plant an outstanding addition to Malaya's young industrial community. Surrounded by a modern housing development, still under construction, the plant is less than a mile from the campus of the new University of Malaya. It is the first completely air-conditioned plant in Malaya.

The new dairy product, a canned sweetened condensed milk, was developed after more than 3 yr. of research in Beatrice Foods' laboratories. The product, which carries a special Beatrice Foods Meadow Gold label, printed in English and Malay, was formulated to suit conditions in Malaya, which has had to import virtually all its dairy products until now. The product fulfills all requirements of natural milk, need not be refrigerated, and remains usable for at least 1 yr.

One of the most progressive, democratic nations in the Far East, the 11-state Federation of Malaya has approximately the same land area as the State of Alabama. It has a population of more than 6,000,000. Principal exports are rubber, tin, coconuts, oil palms, rice, tea, coffee, tapioca, and pineapples.

Accurate profit-control is the job of Mojonnier Milk Testers for standardizing butterfat and total solids content. • Mojonnier Testers have been a must for profitable dairy operation over 45 years. • Fat Determination accurate to .03 of 1%. Total Solids Tests accurate to 1/10th of 1%.

Write for full details today

MOJONNIER BROS. CO.
4601 W. OHIO ST., CHICAGO 44, ILLINOIS

Mojonnier *QUALITY ENGINEERED FOR ECONOMY* **MILK TESTERS**

STANDARD THROUGHOUT THE WORLD

D. L. GRANTHAM has been elected Eastern Regional Vice-President of Beatrice Foods Co. He will supervise all dairy operations for Beatrice Foods districts east from Indiana. He also will continue as president of Meadow Gold Products Corp., 777 Kent Ave., Brooklyn, N. Y., where he will continue to maintain his headquarters, and of Louis Sherry Ice Cream Co., One Little Nassau Street, Brooklyn, N. Y. Both companies are divisions of Beatrice Foods, operating in the Greater New York and New Jersey Metropolitan areas.

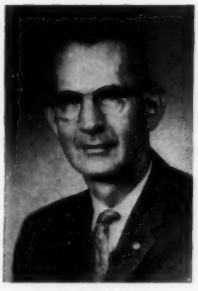
D. L. Grantham

Grantham attended Eastern Illinois College and University of Illinois and is a certified public accountant. He began his career with Beatrice Foods in 1934 in the firm's plant in Mattoon, Illinois. After serving first as office manager, then as sales manager and assistant manager, he was promoted to manager of the Mattoon operations in 1951.

Beatrice Foods Names New Managers

F. C. BAIR has been named manager of the Champaign, Ill., area milk operations of Beatrice Foods Co., and JEFF MCCOLLUM has been promoted to manager of the company's plant at Danville, Illinois.

F. C. Bair



J. McCollum

Bair, manager of the milk plant in Danville for 5 yr., joined Beatrice Foods 26 yr. ago as a special delivery man for the company in Decatur, Illinois, in 1934. Promoted to the office, he advanced to assistant plant superintendent in 6 yr. In 1944, he was named plant superintendent and assistant manager at Decatur. A native of Shelby County, Ill., he

YOU WILL MAKE
Better SOUR CREAM
Better CREAMED COTTAGE CHEESE
Better BUTTERMILK
WITH
PUR-TEST STABILIZER
• PUR-TEST will give your Sour Cream a better body and a satin-smooth texture.
• PUR-TEST inhibits syneresis because of its efficient moisture-retaining properties.
• Samples of PUR-TEST Stabilizer and directions sent on request.
Specializers in Stabilizers
GERMANTOWN
MANUFACTURING COMPANY, 5100 LANCASTER AVE., PHILA. 31, PA.
Warehouses in Seattle, Portland, San Francisco, Houston, St. Louis and Chicago.
Eastern Canadian Distributor: Kingsway Chocolate Co., Ltd., 47 Advance Rd., Toronto 18, Ontario

HOW TO MAKE THE WORLD'S BEST ICE CREAM
You must use the finest dairy ingredients, the finest flavors, the finest mix processing and freezing equipment, the finest packaging—*And The Finest Stabilizer*—KELCO STABILIZER.
Dariloid XL® *for your white mixes*
Sherbelizer® *for your chocolate mixes*
Products of KELCO COMPANY
75 Terminal Ave., Clark, N. J.
20 N. Wacker Drive, Chicago 6, Ill.
530 W. Sixth St., Los Angeles 14, Calif.
Cable Address: KELCOALGIN—CLARKNEWJERSEY

was transferred to Danville as manager in 1955.

A veteran of 25 yr. of experience with Beatrice Foods, McCollum joined the firm as a milk route salesman for the Hoopeston, Ill., branch in 1935. He became manager of the Hoopeston branch in 1947, and, 10 yr. later, was named sales supervisor in charge of all branches of the Danville plant.

Borden Company Acquires a New Subsidiary

United Dairies, Inc., Rio Piedras, Puerto Rico, has been acquired by an unconsolidated subsidiary of the Borden Company. The acquisition involved purchase of United Dairies' stock. The international department of the Borden Foods Company, a division of the Borden Company, has general supervision over Borden's food and dairy operations overseas.

The Company's fluid milk operations in Puerto Rico will eventually be combined in a modern fluid milk processing and distributing plant to be built in the Metropolitan San Juan area. A site will soon be selected, with the plant scheduled to be in operation by July, 1962.

Rumberger Named Cherry-Burrell Branch Manager

D. L. RUMBERGER has been named Manager of the Boston Sales Branch of Cherry-Burrell Corporation, effective immediately.

Rumberger, who was formerly a sales representative at Cherry-Burrell's Pittsburgh Branch, will replace BROOKS JAKEMAN, who has been appointed Industrial Sales Manager–Eastern Region for Cherry-Burrell.

Since 1906—the choice of quality-minded dairy people —

MARSCHALL

The container bearing the Marschall stamp is your assurance of dairy supplies of the highest quality. Specify Marschall Rennet Extract • Rennet Paste and Powder • Cheese Color • Annatto Food Colors • Cottage Cheese Coagulator Tablets • Marlac Culture • Milk-testing Equipment and Supplies

Write for descriptive literature

MARSCHALL
DAIRY LABORATORY, INC.
MADISON 3, WISCONSIN

PREVENT "SLOW STARTERS"

Eliminate lost batches, save time, speed production. Use *Numbered Blends* and the Dairy Laboratories system of rotation that supplies highest quality fresh culture *every week* to keep aroma, body, flavor and production uniform in all fermented milk products.

FLAV-O-LAC FLAKES

Write for details in our free Culture Booklet

DALARE ASSOCIATES

THE DAIRY LABORATORIES
2300 Locust Street
Philadelphia 3, Pennsylvania

Branches: New York • Washington, D.C.

Foremost Dairies Announces New Appointments

R. J. DREWS has been elected Administrative Vice-President of Foremost Dairies, Inc. Before joining Foremost, Drews was Vice-President, Administration, and a Director of Rexall Drug and Chemical Company. He was with Rexall for 14 yr. and prior to that he was

R. J. Drews

W. D. Hudson

associated with the United States Steel Corporation. He is a Certified Public Accountant and was associated with Arthur Andersen & Co. He is a graduate of St. John's University. Drews will be located at Foremost headquarters in San Francisco.

W. D. HUDSON was elected Secretary of Foremost Dairies, Inc. He will continue as head of the Legal Department of the company and is widely known in legal circles. Before joining Foremost in 1959, he was a member of the San Francisco legal firm of Hancock, Elkington and Rothert (now Cushing, Cullinan, Hancock & Rothert). From 1952 to 1956 he served as Deputy Attorney General for the State of California and from 1951 to 1960 he was part-time assistant Professor of Law at Stanford Law School.

Kelco Company Elects Davison

R. O. DAVISON has been elected Vice-President of the Kelco Company, leading manufacturers of refined algin products for the food, paper, drug, brewing, textile, and chemical industries.

Mr. Davison, who has been with the Kelco Company since 1934, is also prominent in the Dairy Industries Supply Association, having recently been re-elected to a second term as president of DISA, which he has previously served in numerous other capacities.

R. O. Davison

Mr. Davison will also continue as Manager of the firm's Eastern Division, with headquarters at 75 Terminal Avenue, Clark, New Jersey. Other Kelco Company offices are located in Chicago and Los Angeles, with extensive research facilities in San Diego.

"The scope of the use of algin and/or algin derivatives continues to widen at such a rate," said Mr. Davison on assuming his new office, "that there will soon be no major industry of any description that does not in some important way utilize these natural extractives from the giant kelp that grows in the coastal waters of southern California."

In addition to the more widely known applications in such fields as foods, pharmaceuticals, and cosmetics, dyestuffs, confectionery, dairy products, and ice cream, Mr. Davison pointed out, there is a rapidly expanding area of general usage including gel applications, emulsions, flocculation aids, vectors for agricultural chemicals, etc.

Mr. Davison is a graduate of Cornell University, class of 1921, where he was an active member of Sigma Phi Society, and prominent in intercollegiate track while studying for his degree in mechanical engineering.

Animal Diseases Spreading

The Council on Public Health and Regulatory Veterinary Medicine of the American Veterinary Medical Association is greatly concerned about the spread of animal diseases. In a resolution adopted April 25 and released on May 5, the Council urged the U. S. Department of Agriculture to protect U. S. livestock against exotic diseases by increasing inspection personnel at ports of entry of foreign animals, and by modernizing inspection and qaarantine facilities.

In its resolution, the Council specifically noted:

The explosive outbreak and spread of African horse sickness in the Eastern Mediterranean area and Southeast Asia. With a mortality rate of about 80% in horses, this disease has spread since late 1959 from Africa to eight countries of the Near East and southeast Asia, causing an estimated loss of 170,000 horses, mules, and donkeys.

The spread of African swine fever to Spain and Portugal. This disease is 100% fatal, causing losses in Spain estimated in excess of 100,000 head, and diminishing the meat supply of Europe.

The appearance of bluetongue in Japan. This debilitating disease of sheep was strictly an African problem until 1924, when it began a rapid and relentless spread through European countries, the Near East, the United States, and now Japan, where great losses are occurring.

The discovery of the African red tick in Florida and New York is alarming because it is a carrier of exotic diseases.

The diagnosis of equine piroplasmasis in a zebra imported into the United States from Africa. Piroplasmosis, caused by a blood parasite, results in anemia.

Dairy Technology Societies

Central Illinois—Joseph H. Petersen, Director of Quality Control, Central Division, The Borden Company, Chicago, spoke at the April 12 meeting at the Jefferson Hotel, Peoria, on Problems in Large-Scale Production of Cottage Cheese.

Ladies' Night was enjoyed May 10 at the Champaign Country Club. The speaker was Michael E. Stiles of South Africa, speaking on Problems of Concern in South Africa and the Congo.

Central Michigan—May 10 meeting was held jointly with the Western Michigan Dairy Technology Society. Judging contests on vanilla

ice cream and on buttermilk were conducted at Anthony Hall on the Michigan State University campus. J. Hoffman Erb, of The Borden Company, presided over the judging and the clinic of vanilla ice cream. Howard Ferriar, of the Dean Milk Company, did likewise for buttermilk and cultured products. Dinner was served at the Holiday Inn of Lansing.

Chicago—Chicago Dairy Technology Society is again offering a $2,000 scholarship, for the 1961 fall semester. The scholarship is paid at the rate of $500 per year for each of the four years the student is in college. For the student to be eligible for this scholarship, his parents must live in Illinois, Indiana, Iowa, Michigan, or Wisconsin. The student winning the scholarship will be permitted to attend the college of his choice, as long as the school carries an accredited curriculum in dairy industry, dairy manufacturing, or dairy technology. Applicants will be screened on the basis of scholastic standing, extracurricular activities, and general character. Applications should be in no later than July 15, 1961. For further information write to Dr. M. D. Bonner, Krim-Ko Corporation, 4830 S. Christiana Avenue, Chicago.

Detroit—April 10 meeting was held at Cregars on Grand River. C. E. Hildreth of Vitex Laboratories in Newark, New Jersey, discussed diet foods and vitamin-mineral fortification of foods in the future.

May 8 meeting was also at Cregars. The speaker was James Driver, Jr., of the Owens-Illinois Toledo plastic sales division, discussing Recent Developments in Plastic Packaging for the Dairy Industry.

Kansas—May 13 featured the annual Ladies' Night dinner meeting at the Elks Club, Wichita. Various types of entertainment, dancing, card playing, visiting, were enjoyed.

Kansas City Area—A double-feature program was given at the dinner meeting, April 27, at Black Angus Restaurant, Kansas City, Missouri. Robert T. Marshall, Instructor in Dairy Husbandry, University of Missouri, Columbia, spoke on Bacterial Growth Inhibitors in Milk and Milk Products; John R. Campbell, Instructor in Dairy Husbandry, University of Missouri, Columbia, on Trends in Milk Production on State and National Levels.

Metropolitan—May 9 meeting was the last meeting of this group until fall. Topic of the evening: Advances in Ultra-High Temperature Pasteurization of Dairy Products, with Dr. William Roberts, Head of Dairy Manufacturing, North Carolina State, as speaker.

Meet the World's Best Judges of Ice Cream . .

Your Customers*

Only your customers will determine whether your special ice cream flavor is a good selection and seller. Will they come back for more? Will they tell others how good it is? They alone, your customers, will judge the goodness and quality. Look for your answers in your sales analysis report.

*with due respect to your flavor panel

Competition is such today that, if you do not offer your customers what they want and like, they will buy elsewhere. You cannot afford to lose that business. To avoid disappointment and to insure a successful and profitable program, let us help you with the many selections we have to offer.

In the interest of the ice cream manufacturer we have developed the unique RAMSEY PLAN FOR MERCHANDISING ICE CREAM which offers you a guide and a wide selection for a successful and profitable program. Our contribution includes the presentation of ice cream samples for your ready-to-taste appraisal.

For full particulars write or call

RAMSEY LABORATORIES, INC.

2742 GRAND AVENUE CLEVELAND 14, OHIO SWeetbriar 1-2727

North Carolina—Topic of the April 12 monthly meeting was The Dairy Industry—Waste Treatment and Stream Pollution. Speaker: Jim Stallings, Superintendent of Waste Treatment, City Health Department, Lexington, North Carolina. Locale: Sedgefield Charcoal Steak House.

For the May 10 meeting George W. Johnson, of the firm of Ingram and Johnson, Architectural Engineers, Charlotte, spoke on Air Conditioning and Ventilation of Dairy Plants.

Philadelphia—Food Fats: Facts and Fantasies was discussed by Dr. Merrill S. Reed, Director of Nutrition Research, National Dairy Council, Chicago, at the April 13 meeting.

May 11 meeting featured Dr. Frank Kosikowski, Professor of Dairy Industry, Cornell University, speaking on Cultured Dairy Products.

Tri-State—April 26 meeting was of interest to ice cream and milk plant operators concerned with practical mechanization. Slides were shown and a talk given by Clyde Ziegler, Branch Engineer, Cherry-Burrell, Pittsburgh, on Throughput, a term associated with automation geared for efficiency.

Western Michigan—May 10 meeting was held jointly with the Central Michigan Society (see account of this under Central Michigan).

PHYSIOLOGY OF REPRODUCTION AND ARTIFICIAL INSEMINATION OF CATTLE

by G. W. Salisbury and N. L. VanDemark

This new study of the reproductive processes of cattle, particularly in the dairy breeds, emphasizes the problems of sterility and fertility as related to artificial insemination. It summarizes and reviews the great mass of data that has accumulated since the practical development of artificial insemination in Europe and the United States. The scope and thoroughness of this book make it invaluable as a text in animal husbandry and as a reference for veterinarians, breeders, researchers, and all professions concerned with animal reproduction.

1961, approximately 700 pages, illustrated, $12.50

REPRODUCTIVE PHYSIOLOGY:
Comparative Reproductive Physiology of Domestic Animals, Laboratory Animals, and Man
by A. V. Nalbandov
1958, 271 pages, illustrated, $6.75

W. H. FREEMAN AND COMPANY
660 MARKET STREET • SAN FRANCISCO 4, CALIFORNIA

FOR TECHNICAL HELP . . .

call the man from Blumenthal

In addition to supplying you with high quality chocolate flavorings and coatings for your ice cream, The Man From Blumenthal will gladly:

1. Discuss prices and prevailing market conditions.
2. Arrange for his laboratory to perform special services that can only be handled by a lab staffed with chocolate technicians and equipped with the latest analytical and pilot plant equipment to produce special types of chocolate for ice cream.
3. Refer your problems to one of our consultants specializing in technical ice cream problems.
4. Conduct research on new ideas suggested by you.

BLUMENTHAL BROS. CHOCOLATE CO.
MARGARET AND JAMES STS., PHILADELPHIA 37, PA.

THE MAGIC TOUCH OF LACTOSE will boost per capita consumption of fluid milk products!

Research at the university and industry level has shown that Edible Lactose, the natural sugar of milk, gives fluid milk products a distinct flavor advantage. Result: realistic product improvements that consumers readily accept. **BUTTERMILK**—Lactose returns the "old fashioned" goodness, tangy aroma and mellow smoothness. It tones down acid sharpness without affecting body development. **SKIM MILK** (modified or fortified)—Lactose builds fuller body and a slightly sweeter taste. Chalky, stale and weak, uninteresting flavors are avoided. **CHOCOLATE DRINK**—Lactose contributes to a richer, more chocolaty taste, smoother body and longer stability. For complete details, get in touch with Western, world's largest producer of Lactose—pure milk sugar. Do it today!

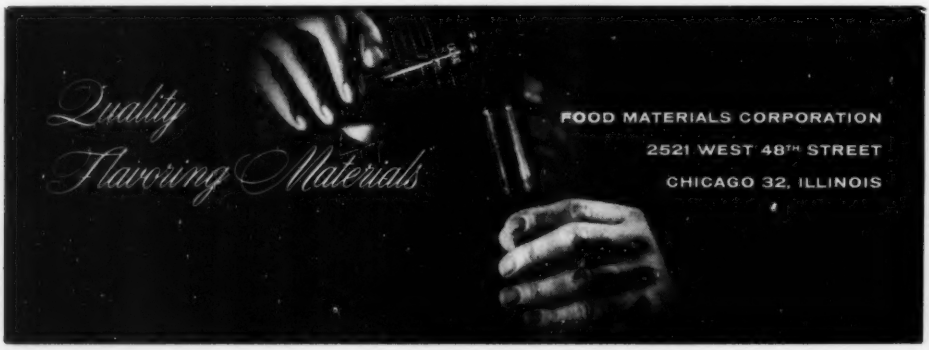
Western Condensing Company • Appleton, Wisconsin

Division of Foremost Dairies, Inc.

lactose

ADVERTISERS' INDEX

Blumenthal Bros. Chocolate Co. 18
Chore-Boy Mfg. Co. 12
Dairy Equipment Company 10
Dairy Laboratories 15
De Laval Separator Co. 8
Difco Laboratories ..... Cover 4
Fiske Associates, Inc. 2
Food Materials Corporation 20
W. H. Freeman and Company 18
Germantown Manufacturing Co. 14
Hubinger Company 4
Kelco Manufacturing Co. 14
Klenzade Products, Inc. 7
Kraft Foods 11
Marschall Dairy Laboratory, Inc. 15
Mojonnier Bros. Company 13
The Nestlé Company, Inc. ..... Cover 3
New Holland Machine Company 6
Pennsalt Chemicals Corp. 3
Ramsey Laboratories, Inc. 17
Western Condensing Company 19
Wisconsin Alumni Research Foundation 5



ANTIBIOTICS IN MILK—A REVIEW [1]

J. L. ALBRIGHT, S. L. TUCKEY, AND G. T. WOODS

Departments of Dairy Science and Food Technology, and the College of Veterinary Medicine, University of Illinois, Urbana

SUMMARY

The widespread use of antibiotics has contributed to the control of diseases and the nutritional well-being of livestock. However, the use of antibiotics in the treatment of mastitis has created problems for the milk processor and consumer. Following treatment of mastitis with antibiotics, they may be found in the milk in sufficient concentrations to inhibit dairy starter microorganisms and cause economic losses to the cheese and fermented milk industries. Penicillin in very small concentrations found in milk may cause reactions in highly sensitive individuals.

Nationwide surveys revealed that penicillin was the primary antibiotic found in the central milk supply. Ten surveys covering a 9-yr. period (prior to 1960) in which 7,201 samples were tested, found 377 (5.2%) to be positive for the presence of antibiotics. The application of testing methods by regulatory and dairy personnel during 1960 resulted in a significant reduction in antibiotic-adulterated milk. Analyses of approximately 770,000 producer milk samples showed an incidence of 0.54%—a tenfold decrease.

When antibiotics are used to treat mastitis, dairymen should follow the prescribed recommendations for withholding milk for human use following treatment. Data compiled on intramammary infusions, intramuscular injections, and oral administration of antibiotics and their vehicles illustrate that wide variations exist concerning the relative persistence of the amount of antibiotics found in milk. The persistence of antibiotics in milk differs in milk from cows in early-, mid-, and late-lactation. Some recent studies using highly sensitive methods indicate that antibiotics are transferred from treated to untreated quarters, but wth penicillin this transfer is slight and of short duration and not likely to present a problem.

When adulterated milk leaves the farm, it is subjected to various processes in the milk plant. Antibiotics in milk are relatively stable to pasteurization temperatures and above, as well as to low temperatures (0-10° F.). Under refrigeration temperatures up to seven days of storage, in raw and pasteurized milk there tends to be a loss in antibiotic activity. Large quantities of milk are necessary to dilute milk from treated quarters, since cultures may be retarded if the concentration of penicillin is approximately 0.05 unit/per milliliter or greater.

Several substances have been found that will inactivate penicillin. The most promising, penicillinase, can be used to hydrolyze penicillin in milk and in penicillin allergy cases.

Larger quantities of inoculum and use of resistant cultures are an aid in the production of cheese made from milk that contains antibiotics.

The presence of antibiotics in milk constitutes an adulteration under the Federal Food, Drug and Cosmetic Act. Educational and testing programs participated in by the Extension Service, veterinarians, dairy inspectors, sanitarians, fieldmen conferences, dairy schools, and government agencies have been helpful and cooperative, but the primary responsibility continues to rest with dairymen.

Antibiotics have been universally used by dairymen and veterinarians for over a decade in the treatment of infectious diseases of dairy cattle. They are administered to dairy cattle via several routes: (1) infusion into the udder

[1] A complete, critical review is beyond the scope of this communication. It is, rather, the purpose of this review to bring together the available literature through the transitional months of 1960. The literature cited herein has been limited to reports concerned with the various phases of the antibiotic problem in milk. Reviews by Overby (134), Marth (115), and Marth and Ellickson (116, 117) also present material in these areas of investigation.

through the streak canal for the treatment of mastitis, (2) injection (intramuscular and intravenous) for the treatment of numerous diseases, and (3) orally for treatment or prevention of diseases or as a dietary supplement. Unfortunately, all of these methods if improperly used can contribute antibiotics and their residues to the central milk supply. Antibiotics in milk are largely the result of improper use of mastitis infusion preparations, or of failure to conform to the instructions on packaged preparations, to the effect that milk from treated quarters be discarded or used for purposes other than human consumption (57, 198).

Milk which contains antibiotics is considered by public health officials to be adulterated and a potential health hazard. This is because certain individuals are allergic to penicillin (48, 65, 192, 209, 210). Furthermore, the consumers of milk and members of the dairy industry also are concerned about the presence and the amount of antibiotics found in milk and its products (5, 24, 28, 32, 33, 39, 40, 56, 62, 81, 85, 96, 99, 101, 105, 198).

Since information is needed by many workers in the dairy field, the following topics will be covered in this review: mode of action of antibiotics; public health problems of antibiotics in market milk; occurrence of antibiotics in market milk and dairy products; antibiotic levels in milk from treated cows; transfer of antibiotics from treated to untreated quarters; and methods for neutralizing antibiotics.

MODE OF ACTION OF ANTIBIOTICS

In general, the action of all antibiotics is the same (54). Antibiotics are either adsorbed onto or absorbed into the cell, and they inhibit the effective growth of the culture in relatively low concentrations. They exhibit some selectivity or differential toxicity under different conditions of use, to a variety of bacterial cells.

It is suspected that antibiotics work directly or indirectly in many possible ways (54): (1) on the cell wall to change its permeability or to cause lysis by increasing osmotic fragility; (2) on one or more enzymatic processes affecting the metabolism in absorption, respiration, energy utilization, synthesis of genetic, structural or metabolic components, detoxication reactions or in maintaining the balance between enzymes, metabolites, and products; (3) on the reproductive processes or chromosomal integrity (failure to produce daughter cells); (4) on excretion processes; (5) on sensitivity mechanisms by increasing or decreasing them; (6) on integrity of defense systems, and (7) on control of homeostasis in any of the other processes considered.

Goldberg (54) has classified antibiotics as (1) surfactants which reduce surface tension and produce cytolysis and cause the formation of (2) antimetabolites, i.e., compounds which prevent the normal functioning of an essential metabolite, usually by combining with the enzyme concerned in its disposition.

Table 1 illustrates the action of penicillin on bacteria.

PUBLIC HEALTH PROBLEMS OF ANTIBIOTICS IN MARKET MILK

The presence of antibiotics in milk constitutes an adulteration under the

TABLE 1

Action of penicillin (54)

Time, minutes (stage of development)	Results
0–2 (Reaction)	Penicillin (up to 0.1 unit/ml) binds irreversibly (137) to all surface penicillin-binding components (PBC) present. Bacterial reproduction ceases.
2–30 (Bacteriostasis)	No death or lag in growth occurs if penicillin is removed. Amount of penicillin bound is doubled with a concomitant loss of internal PBC. Rate of growth is slower after this experience. Helvolic acid prevents permanent damage during this period. Glutamic acid absorption is decreased (53).
30–60 (Bactericidal)	Viable count decreases exponentially during this period. Binding of penicillin continues at a decreasing rate. Cells abruptly cease to accumulate Na^+, K^+, Mg^{++}, $H_2PO_4^-$, glutamate, or gross dry weight. Uptake of CO^{++} and Fe^{++} continues. Removal of penicillin prevents more cells dying, but the lag in growth resumption increases with the duration of penicillin contact. Synthesis of cell protein and cell wall material stops. Synthesis of peptides, phospholipids, and nucleic acids continues.
60–75 (Degeneration)	Nucleic acid (123) and peptide synthesis slow down.
75 (Death)	Cells lose solutes and swell, while phosphate incorporation into large molecules decreases. Cells may burst.

Federal Food, Drug and Cosmetic Act. Their view has been summarized in a policy statement issued November 30, 1955, and reported by Durbin (43) as follows:

"Direct or indirect addition of antibiotic drugs to foods for human consumption: (a) The Food and Drug Administration has received inquiries concerning the use of antibiotic drugs as food preservatives. Careful consideration has been given to this question and the conclusion has been reached that such use constitutes a public health hazard. Consumption of food so treated may cause sensitization of the consumer to such antibiotics and may result in the emergence of strains of pathogenic microorganisms resistant to these drugs. (b) The presence of antibiotic drugs in foods intended for human consumption, or the direct or indirect addition of such drugs to such foods, may be deemed an adulteration within the meaning of Section 402 of the Federal Food, Drug and Cosmetic Act. (c) This statement of policy will not bar the establishment of safe tolerances for antibiotic drugs in or on raw agricultural commodities under the provisions of Section 408 of the Act, where suitable evidence of antibiotic drugs and safety of residues is available."

Public health officials have developed concern along three lines. The first, and possibly most important, has to do with the development of sensitivity reactions to antibiotics. Of the antibiotics that may occur in milk, penicillin is the chief offender in stimulating sensitivity reactions, according to medical authorities (54, 97, 98, 195, 202, 203, 209, 210). However, little information is available on how daily intakes of small amounts of antibiotics affect the population.

Welch (198) sent the results of the 1955 Food and Drug Administration survey to a number of nationally recognized experts in the field of antibiotic therapy, allergy, and pediatrics to obtain their opinions concerning the possible public health significance of the quantities of penicillin found in market milk at that time. Opinions from 31 authorities were to the effect that these concentrations were unlikely to provoke sensitization in a nonsensitive consumer. However, the majority felt that these amounts of antibiotics might cause a reaction in a consumer who was very sensitive. The evidence seems to be that the broad-spectrum antibiotics are poor sensitizers (198). The major concern is with penicillin (54, 97, 98, 202).

Since that time, at least nine cases of allergic dermatitis have been reported to have been caused by penicillin present in milk (46, 192, 209, 210). However, none of the suspected milk samples were analyzed for penicillin and the patients involved were chronic urticaria cases. Welch (198) has mentioned plans to use highly sensitive human volunteers to determine whether the ingestion of milk containing the concentrations of penicillin now found in market milk would cause sensitivity reactions.

Studies of four patients who had previous allergic urticarial reactions from the ingestion of dairy products containing penicillin, disclosed that an injection of 800,000 units of penicillinase, a penicillin-destroying enzyme, rapidly cleared the reaction in 12 to 96 hr., and remained clear for four to seven days. Patients were then able to consume dairy products containing penicillin without further allergic complications, provided they were given prophylactic injections of penicillinase before the ingestion of dairy products (210).

In September, 1956, a medical panel was called to Washington, D. C., to guide the Food and Drug Administration in formulating plans for a program of action to be taken with regard to the problem of antibiotics in market milk. The members of the panel agreed that a small portion of the population (estimated to be about 10%) is susceptible to developing sensitivity to penicillin. This sensitivity ranges from mildly transient to possibly serious reactions. According to the panel, there is no evidence to suggest that those not prone to developing sensitivity to penicillin (about 90% of the population) will be made sensitive by consuming foods containing the antibiotic (198). Kitchen (98) and Weinstein (35) estimated that approximately 5% of the unselected population is allergic to penicillin.

In a nationwide survey of 827 general hospitals and 1,637 physicians, Welch *et al.* (203) studied 2,995 cases of severe reaction to administration of antibiotics. Over 90% of the reaction cases were caused by injections of penicillin, with a fatality rate of 9%. No deaths occurred in cases involving penicillin administered orally.

The second matter of public health concern is that certain infectious bacteria may develop resistance to antibiotics and become refractive to such treatment. For some time, medical authorities have deplored the frequent use of antibiotics for trivial ailments (35, 37, 43, 113). Antibiotics kill large numbers of infectious organisms, but in so doing resistant variants that may not be killed are permitted

to flourish into bacterial populations that are difficult to treat. Some have suggested that unintentional consumption of small amounts of antibiotics in foods might result in the development of resistant microorganisms (37, 43, 113).

A third public health aspect of this problem is the possibility that dairy foods made from milk containing antibiotics may sometimes contain antibiotic-resistant strains of infectious bacteria (33, 37, 195). The findings of Thatcher and Simon (183) have been mentioned in support of this concept. These workers examined 100 samples of cheese obtained from the Canadian retail market and found that 15% of the strains of *Staphylococcus aureus* and 67% of the strains of *Streptococcus* isolated were resistant to penicillin. These resistant strains were probably in the milk at the time the cheese was made, because it is unlikely that the cheese could have been made from milk containing enough penicillin to develop populations of resistant bacteria. The micrococci and streptococci that generally cause mastitis are destroyed by pasteurization techniques. Apparently, much of the cheese that Thatcher and Simon examined was made from raw milk.

Staphylococcal food poisoning has been found in such dairy products as raw milk, dried milk, and cheese (37). It is also known that bovine mastitis caused by staphylococci is common. Furthermore, the indiscriminate use of some antibiotics in the treatment of mastitis has favored the development of strains of staphylococci that are resistant to antibiotics (37, 113). Therefore, there is a need to investigate more thoroughly many aspects of the problem of staphylococci infections in man and animals. More intensive studies of outbreaks should be made when milk or dairy products appear to be vehicles of infection.

Deliberate addition of antibiotics to milk is of infrequent occurrence and the offenders are either ignorant of the consequences or unscrupulous. When antibiotics are used to treat mastitis, dairymen should know how long the milk must be discarded following treatment. The American Dairy Science Association passed a resolution in 1951 that no milk obtained from a mastitis-treated quarter within 72 hr. (six milkings) after the last treatment with this product is to be marketed.

When multiple treatments are used in severe cases of generalized infection and/or cases of mastitis, the 72-hr. period for removal of the milk from the marketable supply may not be sufficient.

The Food and Drug Administration has initiated a three-phase educational program in an attempt to eliminate antibiotics (principally penicillin) from the milk supply (57):

1. *Education of the dairy farmer*—The Cooperative Extension Service (USDA) has an intensive educational program to emphasize the importance of good sanitary and managerial practices. Other educational programs participated in by veterinarians, dairy inspectors and sanitarians, fieldmen conferences, dairy schools, and government agencies can be helpful and cooperative, but the primary responsibility rests with dairymen.

2. *Issuance of a warning statement*—Since July, 1957, a statement warning dairymen to discard milk for 72 hr. after the last treatment has been required to appear on the label of each container of mastitis infusion material sold. On

April 12, 1960, it was proposed to amend the labeling requirements to bear either of the following statements: "Warning: Not for use in dairy animals since this will result in contamination of the milk with the antibiotic." or "Warning: Milk taken from treated dairy animals within.....hours after the latest injection must not be used for human consumption." The blank would be filled in with a figure not greater than 96, based upon data to prove that the milk from treated animals contains no antibiotic residues (49).

3. *Reduction in concentration of penicillin in mastitis preparations*—Prior to August, 1957, some preparations contained up to 1,500,000 units of penicillin per dose. Since that date, the maximum permissible quantity of penicillin per dose is 100,000 units. The smaller quantity of penicillin leaves the udder more quickly and still performs adequate treatment (57). This does not eliminate the possibility of multiple dosages for treatment.

ANTIBIOTICS IN MARKET MILK AND DAIRY PRODUCTS

During the years 1954, 1955, 1956, and 1959, four U. S. surveys were made by the Food and Drug Administration to determine the degree of antibiotic contamination of milk (65, 199, 200). Complete breakdown by individual states was reported in the 1955 and 1956 surveys. Kosikowski (102) recently compiled information on the national incidence of antibiotic residues in milk from January 1 to October 15, 1960—a period of testing and surveillance by dairy and regulatory officials.

Market milk. A summary of the national and local surveys of antibiotic adulteration in the milk supply prior to and after 1960 is presented in Table 2. These surveys cover a ten-year period during which 768,468 samples were tested for the presence of antibiotics. In most cases, penicillin was the primary antibiotic found.

A survey in Pennsylvania published in 1959 revealed that 77% of the dairy farmers were not discarding treated milk for a 72-hr. period (57). The State of Pennsylvania now has a law requiring the testing of all producers' milk once a month for antibiotic residues (24).

The reduction of antibiotic incidence in milk in a large New York State fluid milk and cream dairy firm was accomplished by the application of a steady field test program during 1960. Approximately 10,000 milk samples were tested from 4,000 farms over a 4-mo. period (November, 1959–April, 1960). The presence of antibiotics in milk from producer can farms declined from 6.5% to 0.45-0.52% during this period. On producer bulk tank farms the rate of incidence dropped from 5.1% to between 0 and 0.5%. After the constructive testing program was in effect 2 mo., only one milk tanker from 211 tested was positive, while milk from 203 large milk tankers was negative to antibiotics during this same period (101).

The presence of antibiotics in milk has been reported in Canada (85). Starter cultures were inhibited in milk from 7.3% of 344 herds sampled in the spring of 1952 and 5.4% of 298 herds expressed similar degrees of inhibition in the summer of the same year.

TABLE 2

A. Surveys of antibiotics in U. S. market milk supplies (prior to 1960)

Year	Location	No. samples tested	No. positive	Range (*units/ml*)	Av. conc. (*units/ml*)	% Positive for antibiotics	Type of antibiotic found	References
1951	36 Counties in New York State	1,794	107[a]	0.05–0.5	[b]	6.0	Penicillin	(105)
1951	4 Commercial plants in Champaign-Urbana, Illinois	892	1	[b]	0.15	0.1	Penicillin	(144)
1954	30 Dairy plants in 4 Wisconsin counties	460	20	[b]	[b]	4.3[c]	[b]	(130)
1954[d]	7 Of the Food and Drug districts	94	3	0.01–0.08	0.036	3.2	Penicillin[f]	(199)
1955[d]	All 16 Food and Drug districts[e]	474	55	0.003–0.08	0.015	11.6	Penicillin[g]	(200)
1956[d]	48 States and District of Columbia	1,706	101	0.003–0.55	0.032	5.9	Penicillin[h]	(200)
1956	7 Commercial plants in Columbus, Ohio	151	2	[b]	[b]	1.3	Penicillin	(168)
1958	14 Dealers in Amherst, Massachusetts	122	25	0.021–0.155	0.047	20.5	Penicillin	(171)
1959[d]	All 16 Food and Drug districts	1,170	43	[b]	[b]	3.7	Penicillin	(65)
1959	New York producer dairies							
	A. Cans Nov. 4-Jan. 1	200	13	[b]	0.05	6.5	[b]	(101)
	B. Bulk Tanks Nov. 4-Jan. 1	138	7	[b]	0.05	5.1	[b]	(101)
Total		7,201	377			5.2		

B. National incidence of antibiotic residues in producer milks (January1—October 15, 1960) (102)

1960	17 Dairies throughout the United States	655,763	3,640	[b]	[b]	0.56	[b]	(101)
1960	28 State health and departments of Agriculture throughout the United States	112,705	493	[b]	[b]	0.44	[b]	(101)
Total		768,468	4,133			0.54		

[a] 98/107 samples in April-May and 9/107 occurring during July-August were positive.

[b] Unreported value.

[c] Four and three-tenths per cent of all milks tested contained substances which significantly reduced the activity of starter cultures. When the starter-inhibitory samples were divided on the basis of type of milk plant to which they were delivered, (1) 5.5% of the milk delivered to cheese factories, (2) 3.9% of the milk delivered to fluid milk plants, and (3) 1.3% of the milk delivered to condenseries contained inhibitory substances.

[d] Food and Drug Administration Survey.

[e] Atlanta, Baltimore, Boston, Buffalo, Chicago, Cincinnati, Denver, Kansas City, Los Angeles, Minneapolis, New Orleans, New York, Philadelphia, San Francisco, Seattle, and St. Louis.

[f] One sample contained bacitracin.

[g] One sample contained one of the tetracyclines.

[h] One per cent of all samples tested contained streptomycin, tetracycline, bacitracin, or combinations.

Milk products. Dairy manufacturing plants which are more directly affected than others by the presence of antibiotics in milk are those that produce fermented milk products. Examples of these products are cultured buttermilk, acidophilus milk, Bulgarian milk, sour cream, Cottage Cheese, and all other cheese varieties depending on lactate fermentation. All bacterial organisms which are involved in the production of fermented milk products show varying degrees of inhibition of growth in the presence of the different antibiotics (104) (Table 3).

Milk used for starter cultures. Active starter cultures are the key to successful manufacture of fermented dairy products. Suitable or active cultures can not be maintained if they are inoculated into milk which contains traces of antibiotic residues. Such contaminated milk constitutes a great economic hazard to the dairy plant operator all out of proportion to the amount of milk a single producer would be required to withhold when following withholding recommendations. Hunter (80, 81) has demonstrated that certain sensitive strains of *Streptococcus cremoris* when used as single-strain starter cultures were completely inhibited by 0.07 unit penicillin/ml milk. *Streptococcus lactis* cultures were not as sensitive to penicillin, for 0.1 to 0.25 unit were required to give the same inhibition.

Streptococcus thermophilus, one organism used in Swiss Cheese starters, is very sensitive to the presence of antibiotics. Neal and Calbert (129, 131) selected this as a test organism in the TTC test for antibiotics which can detect 0.04 unit of antibiotics.

Starter cultures appear to be more sensitive to the action of penicillin than other antibiotics, since Bradfield *et al.* (23) demonstrated that mixed lactic starter cultures were not inhibited until 0.25 unit aureomycin/ml milk was present.

Kosikowski and Mocquot (104) have compiled information of the relative sensitivity of cheese starter bacteria to penicillin:

TABLE 3

Critical penicillin levels in milk for bacteria (104)

Bacteria	Penicillin concentrations significantly inhibiting growth
	(I. U. per ml.)
Streptococcus cremoris	0.05–0.10
Streptococcus lactis	0.10–0.30
Streptococci starter	0.10
Streptococcus thermophilus	0.01–0.05
Streptococcus faecalis	0.30
Lactobacillus bulgaricus	0.30–0.60
Lactobacillus acidophilus	0.30–0.60
Lactobacillus casei	0.30–0.60
Lactobacillus lactis	0.25–0.50
Lactobacillus helveticus	0.25–0.50
Lactobacillus citrovorum	0.05–0.10
Propionibacterium shermanii	0.05–0.10

Milk for cheese. Cheese manufacture is dependent on the rate of acid development as well as the total amount produced. If either rate or total quantity of acid is reduced from the optimum, the quality of cheese suffers. Hunter (81) demonstrated that Cheddar cheese made from milk containing 0.1-0.15 unit penicillin/ml milk was criticized as having a fermented flavor and a weak, pasty body after 3 mo. of aging. Bradfield (22) found that when only 0.25-0.31% acidity developed in 7 hr. due to the presence of antibiotics, the cheese did not develop a normal flavor, and the body was weak and pasty. Jacquet (83) reported that when Camembert cheese was made from milk containing 0.5-1.0 unit penicillin/ml milk the cheese was gassy.

Harper studied the phenomena related to noncoagulation of casein in Cottage cheese manufacture when the acidity was at or below the isoelectric point of casein. He showed that when small concentrations of tetracycline-type antibiotics were present in milk there occurred an interaction between casein, the antibiotic, and calcium which prevented clotting of the casein (63).

Milk for other manufactured dairy products. Milk which contains antibiotic residues will contaminate other dairy products, if it is used in their manufacture. If the milk is dried, evaporated, or made into ice cream, the antibiotic is concentrated in these products. Although no manufacturing problems result from the presence of antibiotics in the above-mentioned products, a consumption or use problem is apparent. On the other hand, the Food and Drug Administration was unable to demonstrate antibiotics in evaporated milk, ice cream, powdered milk, and butter samples tested in 1954 (199).

According to Marth and Ellickson (116), Hagen and Ellickson analyzed samples of dried skimmilk and whey for the presence of penicillin. They found from 0.05 to 0.1 unit penicillin/ml of milk in those samples of whey which contained antibiotics. Penicillin-positive samples of dried skimmilk contained between 0.5 and 2.5 units penicillin per gram of product.

Hansen *et al.* (58) showed that sufficient antibiotic residue would be carried into the cream to prevent normal acid development for ripened butter, if the cream was separated from milk containing antibiotics. These workers did not determine whether any antibiotic residue remained in the butter.

Rusoff and co-workers (148) studied the distribution of chlortetracycline in milk and found it to be concentrated in the skimmilk fraction.

ANTIBIOTIC LEVELS IN MILK FROM TREATED COWS

Antibiotics are generally administered to mastitis-infected animals via a solution or suspension manually infused into the infected quarter(s) of the udder. Penicillin was the first of a series of antibiotic preparations employed in mastitis therapy throughout the world (12, 15, 26, 41, 51, 68, 70, 76, 79, 82, 84, 90, 91, 93, 106, 108, 124, 125, 126, 128, 132, 139, 140, 149, 151, 176, 180, 185, 189, 190, 193, 197).

Large amounts of antibiotics are used in the treatment of cows with mastitis. It has been estimated that 75 tons of these drugs are used yearly in the treatment of this widespread infection (200). Approximately 35 companies prepare

products for intramammary treatment that are distributed or sold under 1,500 different labels (24). One pharmaceutical company estimated in 1957 that the dairy industry spends 15 to 20 million dollars a year for these preparations and that at least 80% of their business is in the form of direct sales to farmers (24). Antibiotics are valuable in treating mastitis when properly used; however, there is no indication that the incidence of this disease has been decreased through their promiscuous sale and use (24).

Penicillin is used in the greatest volume, since this drug is effective in the control of streptococci and micrococci, the organisms responsible for most cases of mastitis (27, 41, 68, 124, 139, 180, 185).

Although the use of penicillin injections is still the most popular therapy, other antibiotics have been suggested and used either singly or in combination in the treatment of mastitis. Streptomycin (78, 133, 143, 162, 179), chlortetracycline (16, 23, 92, 121, 143, 148), oxytetracycline (13, 14, 143, 154, 174), bacitracin (17, 133), neomycin (10, 44, 64, 188), polymyxin (64), subtilin (133), chloromycetin (153), and chloramphenicol (133) are now in general use.

According to Marth and Ellickson (116), the quantity of antibiotic used for an individual udder infusion varies according to (1) the type of antibiotic used, (2) the severity of the infection, (3) the frequency with which the antibiotic is to be administered during the course of treatment, and (4) the judgment of the person administering the therapeutic dosage.

Foremilk, mid-milk, and strippings contain different concentrations of antibiotics. Therefore, some of the variation in antibiotic concentrations observed might be due to the portion of the milk assayed (24).

The portion of infused antibiotic which is not absorbed by udder tissues is voided in the milk and the quantity varies from cow to cow. Hovmand *et al.* (74) treated 75 cows by intramammary infusion with 100,000 units penicillin in 25 herds in a dairy district in Denmark. The penicillin excretion of the first milking following treatment was 37.4% of the quantity that was administered. In another dairy district, 100 cows in 23 herds were treated with a similar dosage level and 21.4% of the administered penicillin was found in the first milking following treatment. Wide variations existed between cows within and between herds and districts. For example, with two cows in this study one excreted 2.7% and the other excreted 84% of the amount of antibiotic infused into the udder. Hargrove *et al.* (61) found that 26 to 49% of infused penicillin and 39 to 58% of infused streptomycin was excreted via the milk after treatment. The number of animals treated was not cited.

When S^{35}-labeled penicillin was administered either as an oil or aqueous solution into mastitic udders, the accessibility of the tissues to the drug in both carriers depended primarily upon clear passage in the milk ducts, with diffusion through the tissues being of minor importance. Labeled penicillin in either carrier was usually unevenly distributed, with some regions showing little or no penicillin (189). Similar results were obtained in goats using labeled S^{35} penicillin in oil or aqueous carriers (190). A fairly uniform distribution was

obtained with both carriers when examined 1 and 8 hr. post-injection. Penicillin in oil was concentrated more in the dorsal portions of the udders and was found in the milk ducts rather than in the parenchyma to a somewhat greater degree than was the case for penicillin in aqueous solution (189). These workers postulated that unsuccessful penicillin treatment of mastitis often results from failure of the drug to reach the diseased areas.

The use of emulsified bases are preferred to ointments or oil vehicles for penetration of antibacterial substances into the udder (50, 75, 160, 194). The addition of small quantities of surface-active agents such as iodochlorol (75) to the infusions enhances spreading and penetration of the vehicle.

Udder infusion. Antibiotic concentration in milk from treated cows is summarized and presented in Table 4. It should be noted that wide variations in regard to levels of antibiotics, number of treatments, and length of time from previous treatments exist in these data. Also, some of the investigators tested for antibiotics at lower concentrations than others and, as a result, greater persistence was due in some instances to sensitivity of the test rather than to any difference in the products.

Studies by the Food and Drug Administration in 1953 showed that after the intramammary infusion of one udder quarter with 100,000 units penicillin, the penicillin level for the first and second milking was 8.15 units/ml, for the fifth and sixth milkings 0.042 unit/ml, and no detectable amount on subsequent milkings. The recommendation that milk be discarded for three days following treatment came from this study (5).

Research work reviewed by Plastridge (139) following intramammary infusion of quarters of lactating cows with 100,000 units penicillin in an oily base after 12, 24, 48, and 72 hr. post-infusion were found to vary from 3 to 156, 1 to 25, 0.0 to 3, and 0.0 to 1 units of penicillin per milliliter of milk, respectively.

The highest levels of antibiotics are found in the excreted milk, as one might expect, during the first 24 hr. post-treatment. Also, the use of higher concentrations of antitbiotics results in their presence in the milk for longer periods of time. Jackson and Bryan (82) reported that the amount of milk produced by the cow influences the amount of penicillin found in the milk following treatment. The highest penicillin levels were attained near the middle or end of the lactation period. During the middle of the lactation period, when 300,000 units of procaine penicillin G in oil were injected into the quarter, milk levels of 0.5 unit or more resulted for 144 hr. and at 216 hr. 0.06 unit of penicillin/ml of milk was still present. In cows during their full flow of milk, using the above dosage level, they exhibited levels of 0.06 unit of penicillin/ml of milk at 72 hr. post-treatment.

The length of time penicillin remains in the udder is directly influenced by its carrier. When 100,000 units of penicillin were incorporated into a combination of mineral oil, lanolin derivatives, water, propylene glycol, and nonionic wetting agents, 0.014 to 4 units were present per milliliter 72 hr. post-treatment (51). When cows were treated with propylene glycol, a nonionic wetting agent, and 100,000 units penicillin, the concentration of antibiotics was zero after a

TABLE 4

Penicillin in milk from treated cows via intramammary infusion [1, 2]

Treatment units/infusion [a]	Time *(hr.)* from last treatment	Units/ml milk	References
100,000[b]	72	0.62	(45)
100,000[c]	72	0.54	(45)
100,000	72	0.91	(68)
100,000	72	0.08	(79)
100,000[d]	72	0.65	(173)
100,000[e]	60	0.00	(82)
100,000[f]	72	0.06	(82)
100,000[g]	96	0.06	(82)
100,000[h]	72	0.03	(82)
100,000[i]	120	0.03	(82)
200,000[h]	72	0.03	(82)
200,000[i]	144	0.03	(82)
200,000[g]	96	0.06	(82)
300,000[h]	72	0.06	(82)
300,000[f]	120	0.06	(82)
300,000[i]	216	0.06	(82)
400,000[f]	60	0.06	(82)
500,000	96	0.29	(143)
1,000,000[j]	89	+	(38)

[a] Indicates procaine penicillin G in oil unless otherwise indicated.
[b] Ca penicillin in 5% wax-oil.
[c] Procaine penicillin G in 3% Al-monostearate.
[d] Liquid paraffin suspension with Al-monostearate.
[e] Start of lactation period. Procaine penicillin G in aqueous solution in 10 ml. H_2O.
[f] Middle of lactation period. Procaine penicillin G in aqueous solution in 10 ml. H_2O.
[g] End of lactation period. Procaine penicillin G in aqueous solution in 10 ml. H_2O.
[h] Start of lactation period. Procaine penicillin G in oil with 2% Al-monostearate.
[i] Middle of lactation period. Procaine penicillin G in oil with 2% Al-monostearate.
[j] No tests were conducted after 89 hr. TTC sensitivity test was used (129, 131).
[1] Marth and Ellickson (116) have summarized published data concerning penicillin in milk with emphasis on treatment levels of $< 100{,}000$ units penicillin. Further, they have tabulated published information on streptomycin and chlortetracycline residues following treatment (116).
[2] Brown (24) has compiled published information on the effect of vehicle and dosage level on the persistence of penicillin in milk after one udder infusion.

lapse of 48 hr. (51). Water plus 100,000 units penicillin infused into the mammary gland showed no detectable quantities of penicillin 24 hr. post-treatment (51). Hughes *et al.* (79) reported that procaine penicillin infused into the udder persisted 12 hr. longer than did calcium penicillin. These workers compared procaine penicillin and calcium penicillin (100,000 units per quarter with two injections at 72-hr. intervals) and secured no advantage in favor of either salt against *S. agalactiae* infections. Both salts in the trials produced over 90% bacterological cures.

Streptomycin has been reported in milk for as long as 72 hr. following treatment (133). Chlortetracycline persists in milk of treated cows for as long as 5 wk. (143), with considerable variation existing between cows in the amount of this antibiotic excreted. When oxytetracycline is used in the treatment of bovine mastitis, the persistence of the drug was increased to 54 hr. when three infusions of 800 mg. were administered at 24-hr. intervals (143). Randall *et al.* (143), using 852 mg. oxytetracycline found 1.01 units/ml of milk 96 hr. post-treatment and the milk was free of oxytetracycline by five days post-treatment.

It appears that when moderate to high dosages of antibiotics are administered in the form of one or more tubes per treatment, detectable amounts still occur 72 hr. after treatment.

Intramuscular injections. Data in Table 5 summarize research work in the area of intramuscular injection of penicillin.

Brown (24) recently tabulated published data on the persistence of antibiotics in milk following one intramuscular injection of antibiotics when different salts, vehicles, and dosages were used.

Sadek (149) reported that when penicillin was administered either subcutaneously or intramuscularly to a cow at the rate of 5,000 units per pound of body weight, penicillin was found in the milk at the level of 0.046 unit/ml for 24 hr. Prouty (142) found that penicillin in milk from animals treated intramuscularly with procaine penicillin G in oil (5,000 units/lb) varied in concentration up to 96 hr. with 0.01 unit/ml penicillin present at that time. When cows were injected intramuscularly with penicillin aqueous solution there was a rapid build-up of penicillin in the milk to 0.135 unit/ml 12 hr. post-treatment. By 96 hr. there was less than 0.01 unit/ml of penicillin in the milk (142).

In intramuscular (I.M.) injections of benzathine penicillin V, in a field trial with 27 infected cows, *S. agalactiae* were eliminated in 12 (44%); in three of nine cows (33%) given a single I.M. dose of 6,000,000 units; in five of nine cows (56%) given two I.M. 3,000,000 units doses at 24-hr. intervals, and four of nine cows (44%) treated with 12,000,000 units, given I.M. in three doses of 6,000,000, 3,000,000, and 3,000,000 units at 24-hr. intervals. This organism was eliminated in seven of nine infected cows (77%) treated by udder infusion with 1,000,000 units procaine penicillin G daily for four days (38).

Hollister *et al.* (71) compared normal udders with those that were infected with mastitis. They found that fibrotic quarters produced milk with concentrations of penicillin as high or higher than nonfibrotic quarters. The udder secretions of dry cows after intramuscular injections with 3,000,000 units of benzathine penicillin V showed no penicillin. Findings following the administration of single and multiple intramuscular doses of benzathine penicillin V to 49 cows in seven groups showing udder fibrosis or clinical manifestations of mastitis were reported (71). These workers postulated that ensuing penicillactia results from active participation of the mammary gland, rather than from a mere threshold mechanism of blood circulatory spill. They found penicillin concentration to be low throughout the post-treatment period in all groups, with detectable amounts persisting for 144 hr. ($<$0.01 unit/ml of milk). Benzathine penicillin V was certified by the F.D.A. as reported in the Federal Register (48) with a warning that the milk should not be used for human consumption until seven days post-treatment. This procedure illustrates a point that when accuracy in antibiotic methodology is employed, the product is placed under closer scrutiny.

According to Prouty (142), in a communication from Huebner, "Marketing considerations have resulted in our management's decision to delay production of this compound (benzathine penicillin V) and, thus, at the present time no

TABLE 5

Antibiotics in milk from treated cows via intramuscular injection

Treatment units/injection [a]	Time *(hr.)* from last treatment	Units/ml milk	References
2,000/lb body weight	48	0.000	(207)
2,000/lb body weight[b]	72	0.000	(207)
2,000/lb body weight[f]	72	0.006	(207)
3,000/lb body weight	72	0.000	(20)
3,000/lb body weight[b]	60	0.000	(20)
5,000/lb body weight initially and 2,500/lb at 6-hr. intervals—3×[c]	30	0.080	(203)
5,000/lb body weight	72	0.006	(207)
5,000/lb body weight[b]	72	0.023	(207)
5,000/lb body weight	24	0.046	(149)
5,000/lb body weight[e]	96	0.010	(142)
5,000/lb body weight[d]	96	0.010	(142)
5,000/lb body weight	72	0.005	(143)
5,000/lb body weight[d]	72	0.010	(143)
5,000/lb body weight	72	0.003	(207)
6,000/lb body weight	72	0.010	(20)
6,000/lb body weight[b]	60	0.000	(20)
10,000/lb body weight	72	0.053	(207)
10,000/lb body weight[b]	72	0.009	(207)
10,000/lb body weight[f]	72	0.023	(207)
1,000,000/cow	6	0.000	(197)
3,000,000/cow (mastitis)[e]	72	0.002	(71)
3,000,000/cow (normal)[e]	72	0.009	(71)
3,000,000/cow-3×-24-hr. intervals (mastitis)[e]	72	0.018	(71)
3,000,000/cow-3×-24-hr. intervals (normal)[e]	72	0.055	(71)
3,000,000/cow-3×-24-hr. intervals (normal)[e]	72	0.030	(71)
3,000,000/cow[g]	72	0.000	(70)
3,000,000/cow[f]	72	0.000	(70)
3,000,000/cow[b]	48	0.000	(70)
4,000,000/cow[d]	72	0.000	(69)
6,000,000/cow[e]	72	0.015	(71)
6,000,000/cow[e]	72	0.027	(71)
6,000,000/cow or	12	0.000	(135)
5,000/lb body weight	12	0.000	(2)
10,000,000/cow[h]	48	0.025	(187)

[a] Procaine penicillin G in oil unless otherwise indicated.
[b] Procaine penicillin G (aqueous suspension).
[c] Two different treatments administered 3×.
[d] Procaine penicillin G in oil with aluminum monostearate.
[e] Benzathine penicillin V.
[f] Benzathine penicillin G (aqueous suspension).
[g] Benzathine penicillin V (aqueous suspension).
[h] Dibenzylamine penicillin G (aqueous suspension).

supply of this antibiotic is available for investigational purposes; and no further action is planned for the immediate future."

Oral administration. Henderson *et al.* (66) found antibiotics present in detectable amounts (without giving exact figures) in the blood and milk of the cows which had been fed either 0.5 or 1.0 mg. per pound of body weight level of chlortetracycline. These workers indicated that feeding 0.1 mg. of chlortetracycline per pound of body weight per cow per day did not produce measurable amounts of antibiotics either in the milk or in the blood. This is the dosage permitted for commercial use by the Food and Drug Administration.

Chlortetracycline, at the rate of 130 mg/day, was fed for 60 days to five lactating Holstein cows. The milk samples produced normal acid curd, signifying that the antibiotic was not secreted into the milk or, if present, was of such low concentration that it did not cause any damage in the production of cheese culture (147).

Loosli and Warner (112) reported no antibiotics in the milk when 700 mg. of chlortetracycline was fed daily for ten days. This is slightly more than 0.5 mg. per pound of body weight. Their assay method was not described, and its sensitivity was not indicated. Martin *et al.* (119) indicated that 0.32 mg. per pounds of body weight did not show measurable amounts of chlortetracycline in the milk.

When Shor *et al.* (170) repeated the work of Henderson *et al.* (66), the cows receiving 0.1 mg. per pound of body weight were negative for the presence of the antibiotic in their milk. Slight amounts of chlortetracycline were found in the milk of cows on higher feeding levels (0.5 and 1.0 mg. per pound of body weight), and these slight amounts were eliminated from the milk from all cows by 48 hr. after the feeding of the antibiotic was discontinued.

Sixty Holstein and Jersey cows were involved in reversal trials by Ross *et al.* (146), to determine the effects of feeding 0.1 mg. chlortetracycline per pound of body weight per day for 16 wk. Analyses for chlortetracycline in the milk were negative.

Seventeen feeding trials using oxytetracycline were conducted with 2,370 lactating cows in six states. Cows were paired as to freshening dates and milk production, with one cow as a control and the other receiving 75 to 110 mg. oxytetracycline per day. Average duration of the trials was 186 days. At the level fed, the antibiotic did not appear in the milk when assays were made, nor were the cheesemaking properties affected (114).

Since penicillin salt has been advertised as a safeguard against bloat in cattle, Skaggs and Miller (175) fed two lactating cows procaine penicillin in salt sprinkled over their concentrate feed. No penicillin was found in the milk when the cows received 88.9 mg. (86,700 units) penicillin daily. When the feeding level was increased to 177.8 mg. (178,400 units) daily, approximately 0.05 unit penicillin/ml was found in the milk. A feeding level of 277.8 mg. (270,500 units) daily increased the level in the milk to 0.10-0.15 unit/ml. Blood samples from these cows showed approximately the same level of penicillin content as did the milk samples.

Penicillin was not detected in the milk of two cows following the oral administration of penicillin by Williams and Harold (207). Milk samples were tested twice daily, while penicillin was administered at a level of 1,000,000 units daily for ten days, and 2,500,000 units twice daily for five days. These negative results are not in agreement with the findings of Skaggs and Miller (175).

When penicillin was fed (200 mg/day/cow) as a bloat preventative, no detrimental effects on appetite or milk production were observed, nor did penicillin appear in the milk in measurable quantities (77). Apparently, rapid destruction of antibiotics in the rumen takes place within 3 hr. after feeding (77). This may partially explain why penicillin feeding is effective in the control of bloat at first and then becomes ineffective later.

TRANSFER OF ANTIBIOTICS FROM TREATED TO UNTREATED QUARTERS

Questions have arisen concerning the possible presenec or absence of antibiotics in the nontreated quarters following intramammary treatment of mastitis.

Randall *et al.* (143) reported that milk from the untreated quarters of two cows showed the presence of chlortetracycline (aureomycin) and oxytetracycline (terramycin) following dosages of 426 and 852 mg. of each agent. Chlortetracycline persisted in the milk of the treated quarters for as long as 5 wk. and of the untreated quarters for a period of five days following treatment with the larger dosage levels. Following intramammary infusion of oxytetracyline ointment into two cows, the antibiotic could be detected in the milk from treated quarters for three to four days and from the untreated quarters for one day. When an ointment containing penicillin, dihydrostreptomycin, and sulfonamides was infused into one cow, penicillin could be found in the milk for three days, but dihydrostreptomycin and sulfonamides were present for one day. The untreated quarters were all negative.

Blobel and Burch (21) found that a transfer of oxytetracycline from treated to untreated quarters occurred following intramammary infusion of 426 mg. oxytetracycline per quarter. Of 14 cows used in the trial, the antibiotic was excreted at detectable concentrations through the untreated quarters in one cow after infusion of the right front quarter, in two cows following infusion of the right and left rear quarters, and in three cows subsequent to infusion of the right front, right rear, and left rear quarters. Oxytetracycline levels in milk samples from the untreated quarters of infused cows did not exceed 0.08 unit/ml of milk and were consistently lower than the corresponding blood serum values. When 852 mg. of oxytetracycline per quarter were infused under similar experimental conditions, the antibiotic was found in the milk of untreated quarters of one cow after infusion of the right front quarter, in three cows following infusion of the right front and left rear quarters, and in three cows after infusion of the right front, right rear, and left rear quarters. The respective oxytetracycline levels varied between 0.05 and 0.10 unit/ml of milk. Oxytetracycline levels in blood serum were higher than in milk. The antibiotic was eliminated from the untreated quarters and blood serums within 24 hr. following intramammary infusion of 426 mg. or 852 mg. of oxytetracycline.

A study, in which 11 cows were infused with either 100,000 or 200,00 units penicillin (oil and aqueous carriers) per quarter, showed penicillin present in 21 out of 22 untreated quarters. All untreated quarters were negative for the presence of penicillin by 48 hr. and there were only two positive untreated quarters at 36 hr. post-infusion (8). In another Ohio study, one cow that was infused with 150,000 units penicillin into the right rear and right front quarters shed detectable amounts of penicillin in all four quarters for as long as 84 hr. (7).

An udder infusion study in the right rear quarter of ten cows (three mastitis infected and seven normal cows), using 100,000 units procaine penicillin G in oil and 2 wk. later in aqueous suspension, indicates that penicillin migrated to 14 (out of 60) untreated quarters (135, 136). There was greater penicillin migration when aqueous solutions were used in place of penicillin in oil vehicles—eight out of ten and three out of ten cows, respectively. All untreated quarters were free of antibiotics by the third milking (36 hr.) after udder infusion.

Blobel (19) reported that the transfer of penicillin from treated to untreated quarters occurred in the majority of 17 cows infused with 100,000 units of penicillin G crystalline in a water-in-oil emulsion per quarter. Transfer occurred in 12 cows after infusion of the right front quarters; in 13 cows following infusion of the right front and left rear quarters; and in 14 cows subsequent to the infusion of the right front, right rear, and left rear quarters. By incorporating sensitive methods of testing, penicillin levels in milk samples from the untreated quarters of infused cows ranged from 0.005 to 0.010 unit/ml and were consistently lower than penicillin test levels in the corresponding blood serums. Penicillin was present in both milk and blood up to 30 hr. after treatment. If standard antibiotic field testing procedures (0.05 unit/ml) had been used, it would not have been possible to detect penicillin transfer. Under similar experimental conditions, except that 300,000 units of penicillin G crystalline in a water-in-oil emulsion per quarter were used, measurable amounts of penicillin were transferred to the untreated quarters in all cases (19).

Livoni *et al.* (111) found that penicillin was excreted through the untreated quarters in relatively small amounts in six cows in which the penicillin treatment was limited to one or two quarters at dosage levels of 200,000 units administered three times at 12- and/or 24-hr. intervals, or a single dosage of 400,000 units. This migration from treated to untreated quarters was not considered to be a problem.

A recently completed study on 30 cows receiving insertions from 100,000 to 1,600,000 units of penicillin revealed only two cows with detectable penicillin in a few of the quarters. Amounts detected were approximately 0.03 unit/ml and these values persisted for 24 hr. (100).

Four of 33 cows, given intramammary infusions of antibiotics, had residues in the milk from the untreated quarters. One had antibiotics for one milking, two for two milkings, and one for three milkings. The milk from the treated quarter of the cows in this group contained antibiotic residues from four to 15 milkings. The dosage level was not cited in this study (34).

Penicillin in aqueous or oil suspensions (dosage level not listed) was transferred to untreated quarters in similar amounts in eight cows. Antibiotic concentration in untreated quarters was greatest in low-producing animals and least in higher-producing animals (47).

On the other hand, when Shahani (165) used a green turquoise dye mixed with a penicillin-oil preparation in intramammary infusions for the visual detection of the antibiotic in milk, he found that neither the dye nor the antibiotic diffused from one quarter to another. He postulated that the dye formed a complex with the antibiotic.

Hansen *et al.* (58) infused two cows in one quarter with 100,000 units of penicillin in a physiological salt solution and one cow with 1.0 g. of streptomycin in saline solution, 200 mg. of aureomycin in saline solution, 7.56 g. of sulfanilamide in 20 ml. of oil. Little or no acid development took place in the milk from the treated quarters, regardless of the starter used. Normal acid production developed in the milk from the untreated quarters, indicating that there was no (or a nonsignificant) transfer of antibiotics from treated to untreated quarters.

Following intramammary infusion of one of three different antibiotic treatments (100,000 units penicillin, 0.25 g. dihydrostreptomycin, and 1.0 g. of sulfadimidin; 426 mg. chlortetracycline; or 100,000 units penicillin and 100,000 units dihydrostreptomycin) into the right front quarter of three cows, antibiotics were not detected in the untreated quarters up to 48 hr. post-infusion (94).

In studies by the Food and Drug Administration in 1953, no penicillin was detected in the mixed milk of the other three quarters following an infusion of 100,000 units of penicillin in one quarter of an udder (5).

It is difficult to assess the relative importance and length of time to discard milk from untreated quarters. In the New York control study (101), in which a fifteen-fold decrease in antibiotic contamination of milk resulted, farmers were directed to withhold milk only from treated quarters. It does not appear that the transfer of penicillin to untreated quarters is a problem of serious proportion with the methods of testing now in use.

METHODS FOR NEUTRALIZING ANTIBIOTICS IN THE MILK SUPPLY

The best single method to eliminate the presence of detectable antibiotics in milk, simply stated, is to keep the milk from treated cows out of the central milk supply. With some exceptions, 72 hr. post-treatment is generally long enough; however, Marth and Ellickson (116) have stated that the period of time for elimination of antibiotics from the milk varies as to: (1) kind and concentration of antibiotic therapy used, (2) stage of lactation of the cow, (3) quantity of milk produced, and (4) severity of infection.

Restricted sales of antibiotics for mastitis treatment might reduce antibiotic contamination of milk in some instances. In recognition of this problem, English authorities recently restricted antibiotics for animal use to veterinarians only (6). In Sweden, antibiotics are not only restricted by prescription but the veterinarian and producer are required to report antibiotic treatment of dairy cattle to the dairy processor (6). It makes little difference who administers the

antibiotic, dairyman or veterinarian, if the milk from treated quarters is not withheld for a sufficient period of time.

When contaminated milk leaves the farm, it is subjected to various processes in the milk plant. It has been postulated that certain procedures might reduce the amount of antibiotics before actual consumption by the consumer (116).

Manufacturing processes. The effect of various heat treatments has been summarized and tabulated by Marth and Ellicksen (116). A consensus indicated that penicillin, chlortetracycline, oxytetracycline, streptomycin, and chloramphenicol in milk are relatively stable to pasteurization temperatures and above (116, 163, 167, 169). It would be desirous to know the effects of ultra-high heat treatment of milk for relatively short periods of time on antibiotics present in milk.

When antibiotics were kept at low temperatures (0 to 10° F.) for periods up to 12 wk., the property of the antibiotic to prevent normal growth of lactic starter culture was not changed (67).

Shahani (165) stored milk samples at 39° F. and found losses of 37% for penicillin, 21% for streptomycin, and 15% for chlortetracycline with seven days of storage. It was suggested that bacterial activity of milk enzymes might be responsible for the loss in antibacterial activity (118, 165).

Dilution. One might assume that milk from treated cows when added to the central milk supply would be diluted to the extent that there would not be any difficulty for consumption purposes or in the manufacture of cultured products. Most starter cultures are retarded if the concentration of penicillin is 0.05 unit per milliliter or greater; therefore, milk from one treated quarter could inhibit bacterial action in 250 gal., assuming a normal infusion dosage level of 100,000 units of penicillin with one-half of the potency in the first milking following treatment. Methods for detecting penicillin are capable of detecting 0.003 unit per milliliter or 100,000 units of penicillin in 8,000 gal. of milk (4).

The rapid test for penicillin used by dairy plants and the Food and Drug Administration is sensitive enough to detect 0.05 unit/ml of milk (9), or approximately $\frac{1}{10}$th as sensitive as the overnight test (4).

Hunter (80, 81) found that in order to avoid manufacturing difficulties, assuming the milk was not properly discarded following treatment, not more than 2% of the herds should be treated simultaneously. Overby (134) stated that milk from the first milking after treatment with penicillin should be diluted at least 200 times before being used for cheese-making. Hunter (80, 81) calculated that 90 gal. of milk with five units per milliliter in 4,500 gal. (a five-vat factory supply per day) would provide the critical level of 0.1 unit per milliliter, which would cause appreciable reduction in acid development.

Separation. When whole milk containing antibiotics is separated into cream and skimmilk components, concentrations of either penicillin or streptomycin are equal in the two fractions (58). However, chlortetracycline was concentrated in the skimmilk fraction of treated cows' milk (148).

Inactivation of penicillin. Overby (134) reported that several substances have been found that inactivate penicillin. One of these, hydroxylamine, was

effective but could not be recommended for practical purposes because of its slight toxicity. It was found that taka-diastase and amylase, both derived from an *Aspergillus* strain, were able to inhibit penicillin activity.

Penicillinase, discovered in 1940 by Abraham and Chain (1) in a strain of *E. coli*, hydrolyzes penicillin to the biologically and antigenically inactive penicilloic acid, by opening the beta-lactam ring. Several workers have used this enzyme prior to inoculation with starter cultures (30, 74, 134). Katznelson and Hood (95) found that 0.02 mg. of penicillinase per 100 ml. milk was capable of restoring normal acid development in milk containing 0.5 unit of penicillin per milliliter and 50% acid production in the presence of 1.0 unit per milliliter of milk. Doan (39) and Krienke (110) stated that penicillinase could be used for inactivation in milk, but that its use was not practical because of cost. Overby (134) claims that a Danish preparation, Penase Leo, is inexpensive and the practical use of penicillinase is no longer prohibited by cost.

Increased inoculum for cheese production. Claybaugh and Nelson (32), Katznelson and Hood (95, 96), and Doan (39) recommended heavy inoculation with starter to overcome the effects of penicillin in milk. Krienke (110) pointed out that a second inoculum would be practical only in the presence of low concentrations of the antibiotic. The addition of large amounts of starter to milk for cheese-making will not serve to eliminate any defects produced by penicillin, when at the level of 0.2 unit or more per milliliter (134).

Resistant cultures. Overby (134) developed a single-strain starter that was resistant to three units of penicillin per milliliter and retained this resistance during 20 transfers through milk free from penicillin. Verlinski (191) produced a commercial starter culture which would develop normal acid and flavor in milk containing 0.05 to 0.1 unit of penicillin; but lost its resistance after eight to ten transfers in penicillin-free milk. Hargrove *et al.* (61) adapted cultures of *L. bulgaricus* and *S. thermophilus* to resist three units penicillin per milliliter and *P. shermanii* to 1 unit per milliliter and to 200 mg. streptomycin/milliliter. Johnstone (89) reported some strains exhibited limited resistance to chlortetracycline.

REFERENCES

(1) Abraham, E. P., and Chain, E. An Enzyme from Bacteria Able to Destroy Penicillin. Nature, 146: 837. 1940.

(2) Albright, J. L., Ormiston, E. E., Brodie, B. O., and Witter, L. D. Penicillin in Milk Following Intramuscular and Intramammary Administration of Penicillin in Normal and Mastitic Cows. J.A.V.M.A., 138: 70. 1961.

(3) American Public Health Association. Standard Methods for the Examination of Dairy Products. 10th ed. New York, New York. 1953.

(4) Anonymous. Tentative Methods for the Determination of Antibiotics in Milk. Mimeographed publication, Food and Drug Administration, Revised August, 1958.

(5) Anonymous. Food and Drug Acts on Antibiotics; Dealers Urged to Warn Producers. Milk Industry News, November 8, 1959.

(6) Anonymous. Antibiotics in Milk—Where Do We Stand? J.A.V.M.A., 136: 225. 1960.

(7) Anonymous. Antibiotics and Chemotherapeutics in Milk. Animal Disease Trends, 8(1): 3. 1960.

(8) Anonymous. Antibiotics Residues in Milk. Animal Disease Trends, 8(3): 1. 1960.

(9) ARRET, B., AND KIRSHBAUM, A. A Rapid Disc Assay Method for Detecting Penicillin in Milk. J. Milk and Food Teechnol., 22: 329. 1959.

(10) AYNSLEY, L. H., AND HUGHES, D. L. Neomycin as a Treatment for Bovine Mastitis. British Vet. J., 110: 403. 1954.

(11) BABEL, F. J. Slow Acid Production by Lactic Starters. A Review. J. Dairy Sci., 38: 705. 1955.

(12) BARKER, C. A. V. Observations on the Use of Penicillin in the Treatment of Bovine Mastitis. Canadian J. Comp. Med. Vet. Sci., 9: 235. 1945.

(13) BARNES, L. E. Oxytetracycline in Bovine Mastitis. I. Treatment of Mastitis. Am. J. Vet. Research, 16: 386. 1955.

(14) BARNES, L. E. Oxytetracycline in Bovine Mastitis. II. Milk Levels Following Local and Parenteral Administration. Am. J. Vet. Research, 17: 18. 1956.

(15) BARNUM, D. A. Preliminary Observations on the Use of the Penicillin Bougie in the Bovine Udder. Canadian J. Comp. Med. Vet. Sci., 11: 64. 1947.

(16) BELL, W. B., FLORA, C. C., REAVES, P. M., AND HOLDAWAY, C. W. Aureomycin Concentration in Milk Following Intramammary Infusion and Its Effect on Starter Activity. J. Dairy Sci., 34: 675. 1951.

(17) BENSON, D. V. Treatment of *Streptococcus agalactiae* Mastitis with Bacitracin and Penicillin. J.A.V.M.A., 113: 160. 1948.

(18) BERRIDGE, N. J. Penicillin in Milk. II. The Incidence of Penicillin. J. Dairy Research, 23: 342. 1956.

(19) BLOBEL, H. Concentrations of Penicillin in Milk Secretions and Blood Serums of Cows Following Intramammary Infusion of One or More Quarters. J.A.V.M.A., 137: 110. 1960.

(20) BLOBEL, H., AND BURCH, C. W. Concentrations of Penicillin in Milk of Cows Following Intramuscular Administration. J.A.V.M.A., 136: 477. 1960.

(21) BLOBEL, H., AND BURCH, C. W. Oxytetracycline Concentrations in Blood Serums and Milk Secretions of Cows Following Intravenous or Intramammary Treatment. J.A.V.M.A., 137: 701. 1960.

(22) BRADFIELD, A. The Effect of Mastitis Curatives on Cheesemaking. Canadian J. Comp. Med. Vet. Sci., 14: 127. 1950.

(23) BRADFIELD, A., RESI, L. A., AND JOHNSTONE, D. B. The Presence of Aureomycin in Milk and Its Effect on Cheese Making and Starter Activity. J. Dairy Sci., 35: 51. 1952.

(24) BROWN, R. W. Residues in Milk from Antibiotics Used in Mastitis Treatment. The Nature and Fate of Chemicals Applied to Soils, Plants, and Animals Symposium. Beltsville, Md., ARS 20-9, p. 189. 1960.

(25) BRYAN, C. S. Problems Created for the Dairy Industry by Antibiotic Mastitis Treatments. J. Milk and Food Technol., 14: 161. 1951.

(26) BRYAN, C. S., HUFFMAN, C. F., AND HORWOOD, R. E. A Preliminary Report on Penicillin in the Treatment of Chronic Streptococci Mastitis. Vet. Med., 40:87. 1945.

(27) BYRNE, J. L., PULLIN, J. W., AND KONSK, H. Studies in Mastitis Infection. Canadian J. Comp. Med. Vet. Sci., 10: 16. 1946.

(28) CALBERT, H. E. The Problem of Antibiotics in Milk. J. Milk and Food Technol., 14: 61. 1951.

(29) CERNY, J., AND MORRIS, R. L. A Modified Disc Assay Method for Detecting Antibiotics in Milk. J. Milk and Food Technol., 18: 281. 1955.

(30) CHAPMAN, H. R. The Addition of Penicillinase to Milk of Cows Receiving Penicillin. Dairy Ind., 21: 970. 1956.

(31) CHURCHILL, E. S., DRURY, A. R., LEWIS, B. D., FRANK, C. L., AND BRYAN, C. S. A Report on the Modified Phosphatase Method of Detecting Antibiotic Substances in Milk. Milk Plant Monthly, 40(4): 28. 1951.

(32) CLAYBAUGH, G. A., AND NELSON, F. R. The Effect of Antibiotics in Milk: A Review. J. Milk and Food Technol., 14: 155. 1951.

(33) COLLINS, E. B. Antibiotics in Milk. Paper presented at the Animal Husbandry Livestock Day, Davis, California, February 1, 1958.

(34) COSGROVE, C. J., AND ETGEN, W. M. Antibiotics in Milk. J. Dairy Sci., 43: 1886. 1960.

(35) CRIEP, L. H., ARBESMAN, C. E., SHELDON, J. M., SIEGEL, B. B., AND WEINSTEIN, H. I. The Present Status and Problems Involved in Human Sensitization to Antibiotics Panel Discussion. Antibiotics Ann. 1959-1960. Medical Encyclopedia Inc., New York. p. 979. 1960.

(36) DALGAARD-MIKKELSEN, SV., AND RASMUSSEN, F. Robefarve i Antibiotl Kaholdige Intramammaria Forelobig Meddilelse. Nord. Vet., 9: 852. 1957.

(37) DAUER, C. C., AND DAVIDS, D. J. 1958 Summary of Disease Outbreaks. J. Milk and Food Technol., 22: 335. 1959.

(38) DELLI QUADRI, C. A., MCINTIRE, R. W., PORTER, C. E., AND HAIGHT, R. H. Field Trial Involving Parenteral Use of Benzathine Penicillin V on *Streptococcus agalactiae* Infusion in Two Holstein-Friesian Dairy Herds. J.A.V.M.A., 135: 224. 1959.

(39) DOAN, F. J. Antibiotics in Milk and Discussion of Problems Encountered. Dairy and Ice Cream J., 29: 35. 1950.

(40) DOAN, F. J. Antibiotics in Milk from Farms. J. Dairy Sci., 39: 1766. 1956.

(41) DRURY, A. R. Bovine Mastitis—Diagnosis, Prevention and Treatment. Vet. Scope, 1: 2. 1955.

(42) DUMAIS, R. L'effect muisible de la Penicilline dans ce Lait sur les Ferments lactiques et le Fromage Cheddar. Quebec Lait, 10: 14. 1951.

(43) DURBIN, C. G. Antibiotics in Food Preservation. Am. J. Public Health, 46: 1306. 1956.

(44) DURRELL, W. B. An Evaluation of Neomycin Ointments for the Treatment of Clinical Mastitis in Dairy Cattle. Cornell Vet., 49: 429. 1959.

(45) EDWARDS, S. J., AND HASKINS, M. D. The Determination of Antibiotic Levels in Blood and in Milk Following Parenteral and Intramammary Injections. J. Comp. Pathol. Therap., 63: 53. 1953.

(46) ERSKINE, D. Dermatitis Caused by Penicillin in Milk. Lancet, 1: 431. 1958.

(47) EVANS, D. A., AND STERN, D. N. Observations on the Incidence of Penicillin Transfer from Treated to Untreated Quarters of Cows' Udders Following Infusion of Penicillin for Treatment of Mastitis. J. Dairy Sci., 43: 1886. 1960.

(48) FEDERAL REGISTER. Certification of Benzathine Penicillin V. Federal Register, p. 7614. 1959.

(49) FEDERAL REGISTER. Dairy Animals: Intramuscular or Intravenous Antibiotic Preparations, Labeling. Federal Register, p. 3140. 1960.

(50) FLORESTANO, H. J., BAHLER, M. E., AND JEFFRIES, S. F. Comparative Release of Antibiotics Water-Miscible Type and Grease-Type Ointment Bases. J. Am. Pharm. Assoc. Sci. Ed., 45: 538. 1956.

(51) FOLEY, E. J., STULTS, A. W., LEE, W. S., AND BRYNE, J. L. Studies on Vehicles for Sustaining Penicillin Levels in the Bovine Mammary Gland. Am. J. Vet. Research, 10: 66. 1949.

(52) FOSTER, J. W., AND WOODRUFF, H. B. Microbiological Aspects of Penicillin. I. Methods of Assay. J. Bacteriol., 46: 187. 1943.

(53) GALE, E. F., AND TAYLOR, E. W. Action of Penicillin in Preventing the Assimilation of Glutamic Acid by *Staphylococcus aureus*. Nature, 158: 676. 1946.

(54) GOLDBERG, H. S. Antibiotics—Their Chemistry and Non-Medical Uses. D. Van Nostrand Co., Inc., Princeton, N. J. 1959.

(55) GOYAN, F. H., DUFRENOY, J., STRAIT, L. A., AND PRATT, R. A. Three Hour "Physical Development" Cup-Plate Assay for Penicillin. J. Am. Pharm. Assoc., 36: 65. 1947.

(56) GROSS, K. I. Antibiotics Can Affect—Wn. DHIA Report. November, 1959.

(57) GROVE, D. C. Prevention of Antibiotics in Milk—Present Status. J. Dairy Sci., 42: 199. 1959.

(58) HANSEN, H. C., WIGGINS, G. E., AND BOYD, J. C. Modern Methods of Mastitis Treatment Cause Trouble in the Manufacturing of Fermented Dairy Products. J. Milk and Food Technol., 13: 359. 1950.

(59) HARGROVE, R. E., LEHMAN, R. J., AND MATTHEWS, C. A. Use of Fluorescent Materials for the Indirect Detection of Antibiotics in Milk. J. Dairy Sci., 41: 617. 1958.

(60) HARGROVE, R. E., PLOWMAN, R. D., AND WRIGHT, W. W. Use of Markers in Veterinary Preparations for the Detection of Antibiotics in Milk. J. Dairy Sci., 42: 202. 1959.

(61) HARGROVE, R. E., WALTER, H. E., MALKAMES, J. P., JR., AND MASKELL, K. J. The Effect of Penicillin and Streptomycin on Swiss Cheese Starters. J. Dairy Sci., 33: 401. 1950.

(62) HARMS, J. Crackdown on Penicillin and Pesticides. Hoard's Dairyman, 105: 15. 1960.

(63) HARPER, W. J. Antibiotic Protein Interaction on the Acid Coagulation of Milk. Am. Milk Rev., 22(8): 32. 1960.

(64) HARRIS, A. B., HARRIS, J. R., AND BARR, F. S. A Combination of Neomycin Sulfate and Polymyxin B Sulfate for Bovine Mastitis. J.A.V.M.A., 125: 299. 1954.

(65) HARVEY, J. L. What Can Industry Do to Help Solve Problems of Antibiotics and Insecticides? Milk Industry Foundation Address. Miami Beach, Florida, October 8, 1959.

(66) HENDERSON, B. W., JR., COBBLE, J. W., AND EASTERBROOKS, H. L. The Recovery of Milk and Blood Levels of Aureomycin when Fed to Lactating Dairy Cows. J. Dairy Sci., 40: 617. 1957.

(67) HIBBS, R. A., AND BOYD, J. C. Some Observations on Testing Milk Samples for Antibiotics. J. Milk and Food Technol., 20(4): 109. 1957.

(68) HIGNETT, S. L. Persistence of Penicillin in the Bovine Udder. Vet. Record, 62: 37. 1950.

(69) HOLLISTER, C. J., HUEBNER, R. A., BOUCHER, W. B., AND DE MOTT, T. Bovine Antibiotic Blood Levels Obtained with Antibiotics in Oil Suspensions. Am. J. Vet. Research, 16: 391. 1955.

(70) HOLLISTER, C. J., HUEBNER, R. A., BOUCHER, W. B., AND DE MOTT, T. Parenteral Benzathine Penicillin V in Cattle. Am. J. Vet. Research, 18: 584. 1957.

(71) HOLLISTER, C. J., HUEBNER, R. A., BOUCHER, W. B., AND DE MOTT, T. Parenteral Benzathine Penicillin V in Bovine Mastitis. Am. J. Vet. Research, 20: 287. 1959.

(72) HOOD, E. G., AND KATZNELSON, H. The Effect of Penicillin on the Acid-Producing Ability of Starters. Canadian Dairy and Ice Cream J., 28: 32. 1949.

(73) HORRALL, B. E., AND ELLIKER, P. R. An Activity Test for Cheddar and Cottage Cheese Starters. J. Dairy Sci., 30: 523. 1947.

(74) HOVMAND, H. C., JEPSEN, A., AND OVERBY, A. J. Two Years of Collective Mastitis Campaign in Cooperative Dairies with Practical Control of Penicillin-Induced Starter Failures by Use of Penicillinase or Penicillin Resistant Starter. Nord. Vet. Med., 6: 591. 1954.

(75) HUEBER, W. G., LOFGRIN, C. E., REYNOLDS, W., AND LUTHER, H. G. Vehicles for Intramammary Mastitis Preparations. Vet. Med., 55: 35. 1960.

(76) HUEBNER, R. A., AND BLUMNER, H. N. Bovine Penicillin Blood Levels Obtained with Simultaneous Parenteral Administration of Dibenzylethylenediamine Dipenicillin G and Procaine Penicillin G. Cornell Vet., 44: 56. 1954.

(77) HUFFMAN, C. F. Dairy Feeds and Drug Additives as Related to Cattle Efficiency and Public Health. J. Dairy Sci., 42: 726. 1959.

(78) HUGHES, D. L., AND FARMER, R. K. Studies on Streptomycin in Relation to Its Possible Uses in Veterinary Practice. II. The Absorption, Duration and Excretion of Streptomycin in the Bovine. Vet. Record, 62: 265. 1950.

(79) HUGHES, D. L., CHRISTIE, G. J., AND FARMER, R. K. Procaine Penicillin and Interrupted Penicillin Treatment in *Streptococcus agalactiae* Mastitis. I. Procaine Penicillin in the Bovine Udder. II. Therapy Studies with Procaine Penicillin and Calcium Penicillin. Vet. Record, 62: 11. 1950.

(80) HUNTER, G. J. E. The Effect of Penicillin on Lactic Streptococci. J. Dairy Research, 16: 39. 1949.

(81) HUNTER, G. J. E. The Effect of Penicillin in Milk on the Manufacture of Cheddar Cheese. J. Dairy Research, 16: 235. 1949.

(82) JACKSON, W. F., AND BRYAN, C. S. Penicillin Milk Levels in Cows Following Intramammary Administration. Vet. Med., 45: 395. 1950.

(83) JACQUET, J. La Presence d'Antibiotiques dan le Lait et ses Consequences pour l'Industrie Laitere. Proc. 13th Intern. Dairy Congr., 3: 1143. 1953.

(84) JEPSON, A., AND OVERBY, A. J. The Disturbing Effects of Penicillin Milk in the Dairy Industry and Proposals of Some Technical Methods for the Elimination of Failures. Studies on the Inactivation of Penicillin by Means of Penicillinase. Netherlands Milk and Dairy J., 5: 157. 1951.

(85) JOHNS, C. K. Substances in Herd Milks Inbibiting Acid Production. Canadian J. Agr. Sei., 33: 586. 1953.

(86) JOHNS, C. K. The Action of Penicillin on Lactic Acid Streptococci in Milk. J. Dairy Sci., 39: 913. 1956.

(87) JOHNS, C. K. Applications and Limitations of Quality Tests for Milk and Milk Products. A Review. J. Dairy Sci., 42: 1625. 1959.

(88) JOHNS, C. K. Comments on a New Test for Penicillin in Milk. J. Milk and Food Technol., 23: 6. 1960.

(89) JOHNSTONE, D. B. Effect of Aureomycin upon Acid Production by Cheese Starters. Bact. Proc., 50: 26. 1950.

(90) JORGENSON, S. Syringsforsg med maelk fra penicillinbehandlede dyr. Medl. Dankse Dyrlaegefor., 32: 93. 1949.

(91) KAKAVAS, J. C. Penicillin in the Treatment of Bovine Mastitis. North Am. Vet., 25: 408. 1947.

(92) KANEGIS, L. A., TONELLI, G., AND FALK, H. Aureomycin Milk and Serum Concentrations in Dairy Cows. J. Dairy Sci., 36: 152. 1953.

(93) KASTLI, P. Störungen in der Milchverarbeitung durch die Mastitis—behandlung mit Penicillin. Schweiz. Arch. Tierheilk., 90: 685. 1948.

(94) KASTLI, P. Fragen in Zusammenhang mit einer Penicillinstörung in einer Käserei. Schweiz. Milchztg., Nr. 14. 1957.

(95) KATZNELSON, H., AND HOOD, E. G. Influence of Penicillin and Other Antibiotics on Lactic Streptococci in Starter Cultures Used in Cheddar Cheese Making. J. Dairy Sci., 32: 961. 1949.

(96) KATZNELSON, H., AND HOOD, E. G. Penicillin in Relation to Acid Production in Milk by Starter Cultures Used in Cheddar Cheesemaking. Science, 109: 490. 1949.

(97) KAUTZ, H. D. Penicillin and Other Antibiotics in Milk. J. Milk and Food Technol., 22: 344. 1959. Reprinted from J.A.M.A., 171:49. 1959.

(98) KITCHEN, D. K. Reactions to Penicillin. Am. J. Syphilis, Gonorrhea, and Venereal Diseases, 35: 578. 1951.

(99) KOSIKOWSKI, F. V. Antibiotics in Milk. Am. Milk Rev., 20: 54. 1958.

(100) KOSIKOWSKI, F. V. The Status of Antibiotic Residues in Milk. Proc. 37th Ann. Confer. New York State Assoc. Milk Sanitarians, Syracuse, N. Y., September, 1960.

(101) KOSIKOWSKI, F. V. The Control of Antibiotics in Milk Through a Sound Test Program. J. Milk and Food Technol., 23(9): 285. 1960.

(102) KOSIKOWSKI, F. V. Present National Incidence of Antibiotic Residues in Milk. 88th Ann. Meet. American Public Health Association, San Francisco, Calif., November 2, 1960.

(103) KOSIKOWSKI, F. V., AND LEDFORD, R. A. Tests for Antibiotics in Milk. A Reverse-Phase Disc Assay. J.A.V.M.A., 136: 297. 1960.

(104) KOSIKOWSKI, F. V., AND MOCQUOT, G. Antibiotic Residues in Milk for Cheese. Advances in Cheese Technology. FAO Agr. Studies No. 38. Rome, 1958.

(105) KOSIKOWSKI, F. V., HENNINGTON, R. W., AND SILVERMAN, G. J. The Incidence of Antibiotics, Sulfa Drugs, and Quaternary Ammonium Compounds in the Fluid Milk Supply of New York State. J. Dairy Sci., 35: 533. 1952.

(106) KRIENKE, W. A. The Use of Penicillin to Combat Mastitis. Southern Dairy Prod. J., 46:(6) 32. 1949.

(107) KRIENKE, W. A. Drugs, Mastitis and Acid Starters. Am. Milk Rev., 12(6): 54. 1950.
(108) KRIENKE, W. A. Effects of Various Drugs in Milk from Mastitis-Treated Cows. Southern Dairy Prod. J., 47(5): 37. 1950.
(109) KRIENKE, W. A. Penicillin in Milk. Am. Milk Rev., 11(12): 24. 1950.
(110) KRIENKE, W. A. Effects on Acid Production by Lactic Starters of Various "Drugs" in Milk from Mastitis-Treated Cows. Milk Plant Monthly, 39: 32. 1950.
(111) LIVONI, P., MADELUNG, P., PEDERSEN, J., AND WESTH, J. Fortsatte Syrnings-forsøg Med Maelk Fra Penicillinbehandlede Køer. Nord. Vet. Med., 2: 591. 1950.
(112) LOOSLI, J. K., AND WARNER, R. G. Antibiotics for Dairy Animals. Farm Research, New York State Agr. Expt. Sta., Quart. Bull., 18: 3. 1952.
(113) LOWBURY, E. J. L. Bacterial Resistance to Antibiotics. British Med. Bull., 16: 73. 1960.
(114) MAGRUDER, N. D., LOFGREN, C. E., AND REYNOLDS, W. M. Large-Scale Tests of Feeding Oxytetracycline to Lactating Dairy Cows. J. Dairy Sci., 43: 891. 1960.
(115) MARTH, E. H. Antibiotics in Milk—A Review. I. Recent Developments. II. Methods for Detection of Antibiotics in Milk. J. Milk and Food Technol., 24: 36, 70. 1961.
(116) MARTH, E. H., AND ELLICKSON, B. E. Antibiotic Residues in Milk and Milk Products—A Review. J. Milk and Food Technol., 22: 241. 1959.
(117) MARTH, E. H., AND ELLICKSON, B. E. Problems Created by the Presence of Antibiotics in Milk—A Review. J. Milk and Food Technol., 22: 266. 1959.
(118) MARTIN, J. H., AND HARPER, W. J. Factors Affecting the Loss of Antibiotic Activity in Milk. Antibiotics Ann., 1958-1959. p. 890. Medical Encyclopedia Inc., New York. 1959.
(119) MARTIN, W. H., CLAYDON, T. J., AND BARTLEY, E. E. Aureomycin Content, Bacterial Development, Starter Activity and Cheese Quality of Milk from Cows Fed on Aureomycin Supplement. J. Dairy Sci., 38: 47. 1955.
(120) MATTICK, L. R., ANDERSON, E. O., AND WILDASIN, H. L. A Quantitative Procedure for the Determination of an Inhibitory Substance in Milk—Penicillin. J. Dairy Sci., 38: 829. 1955.
(121) MCCULLOCH, E. C., KISER, J. S., AND MIGAKI, H. Aureomycin in the Treatment of Staphylococcic Mastitis in Cows. Vet. Med., 44: 253. 1949.
(122) MEEWES, K. H., AND MELOSEVIC, S. Suitability of Milk Containing Penicillin for the Manufacture of Camembert Cheese. Milchwirtsch. Forsch., 6: 393. 1954.
(123) MITCHELL, P., AND MOYLE, J. Relationships Between Cell Growth, Surface Properties and Nucleic Acid Production in Normal and Penicillin-Tested *Micrococcus pyogenes*. J. Gen. Microbiol., 5: 421. 1951.
(124) MURNANE, D. A. Second Report on the Treatment of Clinical Streptococci and Staphylococcal Infections of the Bovine Udder with Penicillin. Australian Vet. J., 22: 35. 1946.
(125) MURNANE, D. A. Preliminary Report on the Treatment of Clinical and Subclinical Streptococcal and Staphylococcal Infections of the Bovine Udder with Penicillin. Australian Vet. J., 21: 82. 1945.
(126) MURNANE, D. A. Results of Tests of Newer Penicillin Preparations and of Treatment of Staphylococcal Infections of the Bovine Udder with Penicillin, Sulphone, and Sulphone Plus Penicillin. Australian Vet. J., 25: 85. 1949.
(127) MURNANE, D. A., AND MUNCH-PETERSEN, D. Attempts of Elimination of *Streptococcus agalactiae* from Three Infected Herds by the Use of Penicillin. Australian Vet. J., 35: 242. 1959.
(128) MURPHY, J. M., AND PFAU, K. O. The Value of Local Injections of Penicillin Sodium in the Treatment of *Streptococcus agalactiae* Infection and Mastitis in Cows. Cornell Vet., 35: 88. 1945.
(129) NEAL, C. E., AND CALBERT, H. E. The Use of 2,3,5-Triphenyltetrazolium Chloride as a Test for Antibiotic Substances in Milk. J. Dairy Sci., 38: 629. 1955.

(130) NEAL, C. E., AND CALBERT, H. E. Incidence of Lactic Starter Inhibitory Substances in Individual Herd Milks of Wisconsin. Milk Prod. J., 47(6): 18. 1956.
(131) NEAL, C. E., AND CALBERT, H. E. New Test for Detecting Antibiotics in Milk. Milk Prod. J., 47(6): 54. 1956.
(132) OVAROV, D., AND MUGGLETON, P. W. Penicillin Level in Cow's Milk After Intramammary Infusion of Procaine Penicillin. Vet. Research, 6: 200. 1954.
(133) OVERBY, A. J. The Effect of Various Antibiotics in Milk Following Intramammary Infusion. Nord. Vet. Med., 4: 993. 1952.
(134) OVERBY, A. J. Antibiotics in Milk. Dairy Sci. Abstr., 16: 2. 1954.
(135) ORMISTON, E. E., ALBRIGHT, J. L., WITTER, L. D., AND BRODIE, B. O. Illinois Research on Penicillin in Milk. Illinois Research, 2(4): 7. 1960.
(136) Ormiston, E. E., ALBRIGHT, J. L., WITTER, L. D., AND BRODIE, B. O. Observations on the Infusion of Penicillin in the Mammary Gland of the Bovine. J. Dairy Sci., 43: 1506. 1960.
(137) PASYNSKII, A., AND KASTOOSKOYA, T. The Physical Chemical Action of Penicillin. Biokhimiya, 12: 465. 1947.
(138) PITAL, A., DISQUE, D. T., AND LEISE, J. M. A New Rapid Plate Method for Determining Antibiotic Sensitivity. Antibiotics and Chemotherapy, 6: 351. 1956.
(139) PLASTRIDGE, W. N. Bovine Mastitis: A Review. J. Dairy Sci., 41: 1141. 1958.
(140) PORTER, J. J., CAMPBELL, H. M., WEBER, A. F., AND REUTNER, T. F. Dosages of Penicillin for *Streptococcus agalactiae* Mastitis. J.A.V.M.A., 109: 60. 1946.
(141) PRICE, K. E., ZOLLI, Z., ATKINSON, J. C., AND LUTHER, H. G. Antibiotic Inhibitors. Antibiotics and Chemotherapy, 7: 672. 1957.
(142) PROUTY, C. C. Antibiotic Residues in Milk. 29th Institute of Dairying, Pullman, Washington. March 8, 1960.
(143) RANDALL, W. A., DURBIN, C. G., WILMER, J., AND COLLINS, J. H. Antibiotics Concentration and Duration in Animal Tissue and Body Fluids. I. Blood Serum and Milk of Cows. Antibiotics Ann., 1953-54, p. 421. Medical Encyclopedia, Inc., New York. 1954.
(144) RHOADES, H., AND BOLEY, L. E. Unpublished data. 1951.
(145) ROE, R. S. Chemicals and Antibiotics in Food. Am. J. Public Health, 48: 1498. 1958.
(146) ROSS, R. H., FOURT, D. L., BAILEY, J. W., AND THACKER, D. L. Effect of Feeding Low Levels of Chlortetracycline upon the Health and Milk Production of Dairy Cows. J. Dairy Sci., 42: 1740. 1959.
(147) RUSOFF, L. L., AND HAQ, M. O. Studies on Aureomycin and Vitamin B_{12} Supplementation for Dairy Cows. II. Effect on Production, Composition, and Vitamin B_{12} Content of the Milk. J. Dairy Sci., 37: 677. 1954.
(148) RUSOFF, L. L., LEE, G. C., AND STONE, E. J. Aureomycin (Chlortetracycline) Distribution in Milk. J. Dairy Sci., 40: 1390. 1957.
(149) SADEK, S. E. Penicillin Concentrations in Bovine Blood and Milk After Intramuscular Injection and Its Application in the Treatment of Mastitis. J.A.V.M.A., 125: 387. 1954.
(150) SCHAAF, A. V. D. The Danger of Penicillin in Making Cheese. Friesch. Landb., 46: 388. 1949.
(151) SCHALM, O. W., AND CASSELBERRY, H. H. Treatment of *Streptococcus agalactiae* Infections with Intramammary Infusions of Penicillin. J.A.V.M.A., 109: 470. 1946.
(152) SCHALM, O. W., AND LITTLE, R. B. Treatment of Bovine Mastitis. Bovine Mastitis. McGraw-Hill Company, New York. 1946.
(153) SCHIESSLER, P. Die Wirkung des Chloromycetins bei Eutererkrankugen. Milchwissenschaft, 2: 41. 1952.
(154) SCHIPPER, I. A. Milk, Blood, Urine Concentration of Terramycin Following Intravenous, Intramammary and Intramuscular Administration. Vet. Med., 48: 319. 1953.
(155) SCHIPPER, I. A., AND OLSEN, C. C. Antibiotics in Raw Milk—A Case History in Prevention. J.A.V.M.A., 134: 404. 1959.

(156) SCHIPPER, I. A., AND PETERSEN, W. E. Assay of Antibiotics by Use of Methylene Blue Milk. Am. J. Vet. Research, 15: 475. 1954.
(157) SCHNEIERSON, S. S. Hazards of Antibiotics. Consumers Rpts., 18(4): 162. 1953.
(158) SCOTT, R. Destruction of Starter Activity by Penicillin. Dairy Ind., 15: 1260. 1950.
(159) SCOTT, R. Penicillin and Cheesemaking. Dairy Ind., 16: 440. 1951.
(160) SEEBERG, V. P., AND STREET, J. P. In Vitro Diffusion of Penicillin from Penicillin Ointments for Bovine Mastitis. Vet. Med., 45: 167. 1950.
(161) SEEFEMAN, M., RACKOW, H. G., SCHWARZ, G., AND MUMM, H. Cheese Making Experiments with Milk from Cows Treated with Penicillin. Malk. Kasereiztg., 1: 1012. 1950.
(162) SENZE, A., AND JAKUBOWSKI, S. Influence of Penicillin and Strephomycin on Milk Yield of Cows Excreting *Streptococcus agalactiae* in the Milk, Without Clinical Symptoms. Zeszyt. nauk. wzasz, Sakol. roin., Wroclau Nr., 10: 135. 1957. (Also in Dairy Sci. Abstr., 21: 166. 1959.)
(163) SHAHANI, K. M. The Effect of Heat and Storage on the Stability of Aureomycin in Milk, Buffer, and Water. J. Dairy Sci., 40: 289. 1957.
(164) SHAHANI, K. M. Factors Affecting Terramycin Activity in Milk, Broth, Buffer, and Water. J. Dairy Sci., 41: 382. 1958.
(165) SHAHANI, K. M. Visual Detection of Antibiotics in Milk by Means of a Dye. Antibiotics Ann. 1958-1959. p. 883. Medical Encyclopedia, Inc., New York. 1959.
(166) SHAHANI, K. M., AND BADAMI, B. C. A Resazurin Disc Assay Method for Detecting Antibiotics and Natural Starter Inhibitory Activity in Milk. J. Dairy Sci., 41: 1510. 1958.
(167) SHAHANI, K. M., GOULD, I. A., WEISER, H. H., AND SLATTER, W. L. Effect of Heat Treatment on Streptomycin and Chlortetracycline. Antibiotics Ann., 1954-1955. p. 353. Medical Encyclopedia, Inc., New York. 1955.
(168) SHAHANI, K. M., GOULD, I. A., WEISER, H. H., AND SLATTER, W. L. Observations on Antibiotics is a Market Milk Supply and the Effect of Certain Antibiotics on the Keeping Quality of Milk. Antibiotics and Chemotherapy, 6: 544. 1956.
(169) SHAHANI, K. M., GOULD, I. A., WEISER, H. H., AND SLATTER, W. L. Stability of Small Concentrations of Penicillin in Milk as Affected by Heat Treatment and Storage. J. Dairy Sci., 39: 971. 1956.
(170) SHOR, A. L., JOHNSON, W. P., AND ABBEY, A. Effects of Various Amounts of Chlortetracycline in the Rations of Lactating Dairy Cattle. J. Dairy Sci., 42: 1203. 1959.
(171) SIINO, F. A., CZARNECKI, R. B., AND HARRIS, W. K. The Incidence of Penicillin in the Market Milk Supply of a Local New England Area. J. Milk and Food Technol., 2: 211. 1958.
(172) SILVERMANN, G. J., AND KOSIKOWSKI, F. V. Systematic Testing of Inhibitory Substances in Milk. J. Milk and Food Technol., 15: 120. 1952.
(173) SIMESEN, B. Penicillin Preparations for Intramammary Application. Nord. Vet. Med., 11: 523. 1959.
(174) SIMON, J., AND SCHMIDT, E. G. Terramycin in the Treatment of *Streptococcus mastitis*. Vet. Med., 48: 61. 1953.
(175) SKAGGS, S. R., AND MILLER, D. D. Penicillin Levels in Milk from Lactating Cows Fed Various Amounts of Procaine Penicillin. J. Dairy Sci., 42: 1742. 1959.
(176) SLANETZ, L. W., AND ALLEN, F. E. Penicillin Therapy in Streptococcal Mastitis. J.A.V.M.A., 111: 125. 1947.
(177) SLATTER, W. L. Detecting the Presence of Antibiotics in Pasteurized Milk. The Milk Dealer, 43: 60. 1954.
(178) SMITASIRI, T., KOSIKOWSKI, F. V., GUTHRIE, R. S., AND FINCHER, M. G. Dyes as Markers for Antibiotic-Contaminated Milks. J. Milk and Food Technol., 21: 255. 1958.
(179) SMITH, C. R., PETERSON, W. E., AND BROWN, R. W. Effect of Infused Streptomycin in the Mammary Gland. Proc. Soc. Exptl. Biol. Med., 68: 216. 1948.

(180) SPENCER, G. R., KRAFT, M. E., AND UNDERBJERG, G. K. L. Efficiency of Intramammary Infusion with Penicillin and Diluents on *Streptococcus mastitis*. Am J. Vet. Research, 8: 325. 1947.

(181) STEVENSON, W. G. Injury as a Cause of Mastitis. Canadian J. Comp. Med. Vet. Sci., 10: 115. 1946.

(182) STORRS, F. C., AND HIETT BROWN, W. The Incidence of Penicillin In Milk Supplies. J. Dairy Research, 21: 337. 1954.

(183) THATCHER, F. S., AND SIMON, W. The Resistance of Staphylococci and Streptococci Isolated from Cheese to Various Antibiotics. Canadian J. Public Health, 46: 407. 1955.

(184) THOMAS, S. B., AND PANES, J. J. Further Studies on the Effects of Penicillin in Milk Used for the Manufacture of Cheshire Cheese. J. Soc. Dairy Technol., 9: 87. 1956.

(185) THORP, W. T. S., UHRICK, I. J., AND STRALEY, E. J. Concentrations of Penicillin in the Bovine Mammary Gland Following Infusion and Penicillin Tolerance of Certain Streptococci. Am. J. Vet. Research, 8: 157. 1947.

(186) TREMBATH, R. H. Effect of Penicillin in Milk on Starter Cultures. Australian J. Dairy Technol., 5: 143. 1950.

(187) TUCKER, E. W., AND FULLER, R. W. Blood and Milk Levels Following Intramuscular Injection of Dibenzylamine Penicillin G and the Effect on *Streptococcus agalactiae* Infection of the Bovine Udder. Antibiotics Ann., 1953-1954. p. 433. Medical Encyclopedia, Inc., New York. 1954.

(188) TUCKER, E. W., AND JOHNSON, S. D. Observations on Treatments of Udder Infections and Clinical Mastitis with Neomycin Sulfate. J.A.V.M.A., 123: 332. 1953.

(189) ULLBERG, S., HANSSON, E., AND FUNKE, H. Distribution of Penicillin in Mastitic Udders Following Injection—An Autoradiograph Study. Am. J. Vet. Research, 19: 84. 1958.

(190) ULLBERG, S., HANSSON, E., AND FUNKE, H. Distribution of Aqueous Penicillin and Penicillin in Oil in Normal Goat Udders Following Intramammary Injection—An Autoradiograph Study. Am. J. Vet. Research, 19: 135. 1958.

(191) VERLINSKI, S. Penicillin-Resistant Starter. Proc. Intern. Dairy Congr., 2: 594. 1959.

(192) VICKERS, H. R., BAGRATUNI, L., AND ALEXANDER, S. Dermatitis Caused by Penicillin in Milk. Lancet, 1: 35. 1958.

(193) VIGUE, R. F. Relative Efficacy of Three Antibiotic Combinations in Bovine Mastitis. J.A.V.M.A., 124: 377. 1954.

(194) VIGUE, R. F. A Practical Mastitis Infusion Additive. Vet. Med., 54: 67. 1959.

(195) WAISBREN, B. A., AND STRELITZER, C. L. Antibiotic Sensitivities of Staphylococci Isolated Before and After Patients Were Given Antibiotics. Am. J. Med. Sci., 238: 202. 1959.

(196) WALKER, L. J. Some Problems in Cheese Production. J. Soc. Dairy Techlol., 12: 37. 1954.

(197) WATTS, P. S., AND MCLEOD, D. H. The Estimation of Penicillin Blood Serum and Milk of Bovines After Intramammary Infusion. J. Comp. Pathol. Therap., 56: 170. 1946.

(198) WELCH, H. Problems of Antibiotics in Food as the Food and Drug Administration Sees Them. Am. J. Public Health, 47: 701. 1957.

(199) WELCH, H., JESTER, W. R., AND BURTON, J. M. Antibiotics in Fluid Milk. Antibiotics and Chemotherapy, 5: 571. 1955.

(200) WELCH, H., JESTER, W. R., AND BURTON, J. M. Antibiotics in Fluid Market Milk. Antibiotics and Chemotherapy, 6: 374. 1956.

(201) WELCH, H., LEWIS, C. N., KERLAN, I., AND PUTNAM, L. E. Acute Anaphylactoid Reactions Attributable to Penicillin. Antibiotics and Chemotherapy, 3: 891. 1953.

(202) WELCH, H., LEWIS, C. N., WEINSTEIN, H. I., AND BRECKMAN, B. B. Severe Reactions to Antibiotics. A Nationwide Survey. Antibiotics Ann., 1957-1958. p. 296. Medical Encyclopedia, Inc., New York. 1958.

(203) WELSH, M., LANGER, P. H., BURKHARDT, R. L., AND SCHROEDER, C. R. Penicillin Blood and Milk Concentrations in the Normal Cow Following Parenteral Administration. Science, 108: 185. 1948.

(204) WHITEHEAD, H. R., AND COX, G. A. The Detection of Penicillin in Milk. J. Appl. Bacteriol., 19: 247. 1956.

(205) WHITEHEAD, H. R., AND LANE, D. J. The Influence of Penicillin on the Manufacture and Ripening of Cheddar Cheese. J. Dairy Research, 23: 355. 1956.

(206) WILKOWSKE, H. H., AND KRIENKE, W. A. The Influence of Penicillin on the Lactic Acid Production of Certain Lactobacilli. J. Dairy Sci., 34: 1030. 1951.

(207) WILLIAMS, W. W., AND HAROLD, L. C. Antibiotic Residues in Milk After Parenteral and Oral Administration in Cows. J.A.V.M.A., 137: 525. 1960.

(208) WITTER, L. D., AND TUCKEY, S. L. Variable Factors in the New Test for Penicillin in Milk. J. Milk and Food Technol., 23: 230. 1960.

(209) ZIMMERMAN, M. C. Penicillinase Treatment of Fifty-two Patients with Allergic Reactions to Penicillin. Antibiotics Ann., 1957-1958. p. 312. Medical Encyclopedia, Inc., New York. 1958.

(210) ZIMMERMAN, M. C. Chronic Penicillin Urticaria from Dairy Products, Proved by Penicillinase Cures. Arch. Dermatol., 79: 1. 1959.

RELATION OF MILK SERUM PROTEINS AND MILK SALTS TO THE EFFECTS OF HEAT TREATMENT ON RENNET CLOTTING[1]

A. KANNAN[2] AND ROBERT JENNESS

Department of Agricultural Biochemistry, University of Minnesota, St. Paul

SUMMARY

A study was made of the effects of heat at 85 or 90°C. for 30 min. on the rennet coagulability of skimmilk and of artificial systems composed of caseinate centrifuged from skimmilk and dispersed in milk dialysate with and without added milk serum proteins. Such heat treatments of skimmilk cause an immediate prolongation of the rennet clotting time at 35°C. and a further prolongation (hysteresis) when the heated sample is held following heating. These effects occurred with the artificial systems only if they contained β-lactoglobulin. Furthermore, 0.2% β-lactoglobulin added to skimmilk enhanced the magnitude of both the immediate prolongation and the hysteresis. Thus, heat treatment appears to alter β-lactoglobulin or to cause it to complex with casein in such a way that it inhibits rennet clotting. The rate of action of rennet at 16°C. is slower on heated than on raw milk, hence the heat-induced reactions involving β-lactoglobulin appear to interfere with the primary (enzymatic) action of rennet.

Heat treatment of eight samples of skimmilk at 85°C. for 30 min. caused an average transfer of 6 mg. of calcium and 4 mg. of phosphorus per 100 ml. from the dissolved to the colloidal state. The dissolved calcium and phosphate tended to return to their original concentrations when heated milk was held cold. Dialysis of heated milk against a large volume of raw milk increased the colloidal phosphate and partially restored the rennet coagulability. These changes in state of calcium and phosphate occurred in the absence of β-lactoglobulin and were not enhanced in magnitude by added (0.2%) β-lactoglobulin. Thus, they seem to be independent of that protein. The hysteresis phenomenon may result from loss of colloidal calcium phosphate from the caseinate particles on holding after heating.

It is well known that heat treatment of milk increases its rennet clotting time. Not only is there an immediate increase in rennet clotting time after heating in the range of 65-100° C. for times up to 30 min., but a further increase occurs if the sample is held after heating. These effects were demonstrated in 1929 by Mattick and Hallett (9) and corroborated by Powell (13), Powell and Palmer (14), and Pyne (15). Pyne (15) demonstrated that the change on aging (referred to as hysteresis) coincides with the release of calcium and phosphate from the colloidal particles. Hilgeman and Jenness (5) reported that the dissolved calcium and phosphate of skimmilk decrease upon heating and revert to nearly the original levels upon subsequent holding at 0-5° C. Since both heating and subsequent aging prolong rennet clotting time but have opposite effects on dissolved calcium and phosphate, the changes in clottability cannot be explained simply or solely on the basis of shifts in the equilibrium between dissolved and colloidal calcium phosphate.

Powell and Palmer (14) were able to produce both the immediate effect of heat and the hysteresis in artificial systems of calcium-caseinate-phosphate. Pyne (15) also observed the hysteresis in such systems. These findings suggest

Received for publication August 26, 1960.

[1] Paper No. 4464, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Present address: Department of Health, Delhi Municipal Corporation, Delhi, India.

that the calcium-caseinate-phosphate is solely responsible for these effects. On the other hand, a possible involvement of the serum proteins is indicated by the finding of Moir (11, 12), that both the immediate prolongation of rennet coagulation time by heat treatment and the hysteresis are accompanied by small decreases in the nitrogen content of rennet whey. Moir employed heat treatments of 73.9 and 85° C. for 30 and 60 sec. The work reported in this paper was undertaken to ascertain whether the milk serum proteins actually are involved in these phenomena and if so, how their effects are related to the shifts in state of calcium and phosphate.

MATERIALS AND METHODS

Skimmilk was separated in the laboratory from raw mixed milk secured either from the University herd or from a commercial bulk supply.

Caseinate systems consisted of caseinate centrifuged from raw skimmilk and dispersed in an equilibrium milk dialysate, prepared by dialyzing distilled water in a cellophane sack vs. at least 30 volumes of skimmilk or whole milk for 48 hr. at 0-5° C. The caseinate was collected by centrifuging raw skimmilk at 13,000 r.p.m. for 70 min. at room temperature in cellulose nitrate tubes (10 by 75 mm.) in a Servall angle centrifuge, Model SS 1-A. This procedure resulted in separation of a caseinate gel and a clear supernatant whey. Analyses indicated that at least 90% of the caseinate was sedimented. The caseinate gel was washed by dispersing it in equilibrium dialysate and recentrifuging. It was then redispersed in dialysate. A level of 0.1% serum protein or less was attained in the resulting dispersion at a normal casein level of 2.5%. The composition of these sols usually was controlled volumetrically (i.e., they were made up with dialysate to a volume about 10% less than that of the original skimmilk centrifuged). In some cases, the original skimmilk and the sol were analyzed for nitrogen distribution by the Rowland method (7) and the latter was adjusted to exactly the casein (and in some cases the serum protein level) of the former.

The serum protein mixture added in certain experiments was prepared by acidifying fresh skimmilk to pH 4.6 with acetic acid, dialyzing, and freeze-drying. The individual serum proteins used were β-lactoglobulin recrystallized four times according to Larson and Jenness (8), α-lactalbumin recrystallized twice according to Gordon and Ziegler (4), bovine blood serum albumin (Armour), and eu- and pseudoglobulin prepared from colostrum according to Smith (16).

All artificial sols were equilibrated by dialysis against skimmilk overnight at 0-5° C. before being subjected to heat treatment and rennet clotting tests. The heat treatment was usually 85° C. for 30 min., but in a few cases 90° C. for 30 min. was used. The sample was cooled quickly to 35° C. for the rennet clotting test, which was performed within 5 min. after the cessation of heating. To determine the effects of the hysteresis, additional tests were made after holding the samples for 3 hr. at 35° C., or in some cases after storage overnight at 0-5° C.

The apparatus used for determining rennet clotting time was similar to that described by Berridge (1) but on a smaller scale. It consisted of a 10- by 100-mm. test tube which could be attached by a stopper and shaft to a motor which rotated it at 3 r.p.m., at an angle of about 40° to the horizontal in a water bath at 35° C. In performing the clotting test, 1 ml. of the sample and 0.1 ml. of a 1:10 dilution [3] of commercial rennet extract were quickly tempered to 35° C. (if not already at that temperature) in separate tubes. The sample was quickly poured into the tube containing rennet, which was then attached to the stopper and rotated. The clotting time was measured with a stop watch from the mixing of sample and rennet to the first appearance of flocculation. In general, the raw milks gave clotting times of 30-40 sec. with the rennet employed. This could be easily replicated within 1 sec. in separate determinations.

Ultrafiltrates for analysis were prepared with the pressure apparatus described by Tessier and Rose (18). Calcium was determined by the method of Jenness (6), and phosphorus by that of Sumner (17).

RESULTS

Rennet coagulation of caseinate sols. To determine whether the centrifugation and redispersion had any influence on the stability of the caseinate system, a number of samples of skimmilk were centrifuged and the separated caseinate was redispersed immediately back into the supernatant whey. The results of this experiment, shown in Table 1, indicate that such systems respond to

TABLE 1

Effect of heat treatment (85° C. − 30 min.) on rennet clotting time of skimmilk and caseinate dispersions in centrifuge whey

	Rennet clotting time (sec.)								
	Skimmilk			Casein in raw whey			Casein in heated [a] whey		
		Heated			Heated			Heated	
Lot	Not heated	Immed.	3 hr. at 35° C.	Not heated	Immed.	3 hr. at 35° C.	Not heated	Immed.	3 hr. at 35° C.
2	54	65	56	40	90	78	....	....	....
3	40	99	110	45	100	197	55	58	72
4	37	65	80	41	78	98	59	56	62
7	34	65	95	44	103	125	....	....	....

[a] Centrifuge whey heated at 85° C. − 30 min. before recombining with caseinate.

heating comparably to skimmilk. However, in two cases the supernatant whey was heated at 85° C. for 30 min. before the caseinate was redispersed in it. When this was done, the effect of subsequent heat treatment on the rennet clotting was virtually eliminated. Thus, this preliminary experiment suggests that some constituents of the serum are involved in the effect of heat upon the rennet clotting time.

Eleven experiments were next performed with caseinate dispersed in equilibrium dialysate with and without added serum protein. Table 2, summarizing

[3] A 1:20 dilution was used in the experiments involving incubation at 16° C.

TABLE 2

Effect of heat treatment (85° C. – 30 min.) and presence of serum proteins on rennet clotting time of skimmilk and dispersions of caseinate in milk dialysate

	Rennet clotting time (sec.)											
	Skimmilk			Caseinate in dialysate [b]			Caseinate in dialysate + serum protein [c]			Caseinate in heated (dialysate + S.P.) [c, d]		
Lot	Not heated	Heated immed.	3 hr.[a]	Not heated	Heated immed.	3 hr.[a]	Not heated	Heated immed.	3 hr.[a]	Not heated	Heated immed.	3 hr.[a]
1	37	80	94	45	35	36	21	147	205	40	44	45
2	54	65	56	37	25	17	33	108	90	73	58	40
3	40	99	110	35	38	43	42	105	180	54	45	57
4	37	65	80	31	34	30	43	200	210	60	45	51
5	39	74	97	40	38	34	34	600	900	....	....	....
6	41	85	105	33	29	34	34	185	195	....	....	....
7	34	65	95	31	29	29	31	270	340	....	....	....
8	39	105	....	32	35	....	30	65	....	....	....	....
9	40	80	100	21	20	23	....	....	....	....	....	....
10	38	65	104	20	22	23	25	180	155	....	....	....
11	35	130	....	28	38	....	24	183	....	....	....	....

[a] Held 3 hr. at 35° C. after heating.
[b] About 0.1% serum protein.
[c] About 0.5% serum protein.
[d] Dispersion of serum protein in dialysate heated at 85° C. for 30 min. before recombining with caseinate.

these data, shows that caseinate dispersed in dialysate had a comparable clotting time to that of skimmilk but that it did not respond to heat treatment like skimmilk. However, when serum protein was added, the system responded to heating in a manner at least qualitatively similar to the behavior of skimmilk. Both the immediate increase in clotting time and the hysteresis effect were usually observed with such systems. [Lot 2 is an exception, in that hysteresis did not occur in the heated skimmilk, the caseinate dispersed in whey (Table 1), or the caseinate dispersed in dialysate plus serum protein (Table 2).] If the serum protein was dispersed in the dialysate and heated before adding the caseinate, the effect did not occur. This latter result is similar to that observed with the heated supernatant whey in Table 1.

The next experiment was designed to determine the effect of the serum protein level on the response to heat treatment. A sol of caseinate dispersed in equilibrium dialysate was prepared and graded levels of serum protein were added to separate portions of it. As Figure 1 shows, the rennet clotting time

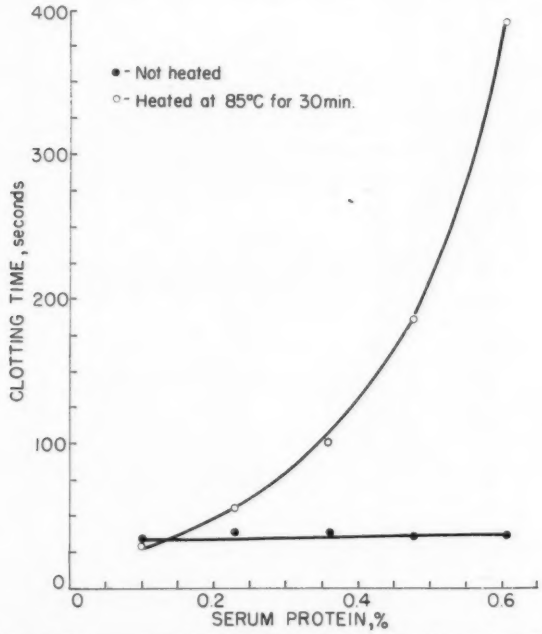


FIG. 1. Influence of serum protein level on rennet clotting time of caseinate dispersions.

of the heated systems increased as the serum protein content was raised. In replicate experiments, the effect of the level of serum protein on rennet coagulation time was found to be slightly more or less pronounced than indicated in Figure 1. The various levels of serum protein had no effect on the rennet clotting of the unheated systems.

Since the hysteresis effect is very marked in samples aged at 5° C., a series of sols similar to those used in the previous experiment were held for 24 hr. at this temperature after being heated. The data (Table 3) support those in

TABLE 3
Effect of heat treatment and aging on rennet clotting time of skimmilk and caseinate dispersions in milk dialysate

Lot		Rennet clotting time (sec.)					
		Skimmilk		Casein in dialysate		Casein in dialysate + serum protein	
		Not aged	Aged 24 hr. at 5° C.	Not aged	Aged 24 hr. at 5° C.	Not aged	Aged 24 hr. at 5° C.
9	Unheated	40	43	21	26	....	....
	Heated 85° C. — 30 min.	80	400	20	57	....	....
10	Unheated	38	45	20	20	25	25
	Heated 85° C. — 30 min.	65	250	22	38	180	230
11	Unheated	35	....	28	....	24	....
	Heated 85° C. — 30 min.	130	420	38	70	183	310

Tables 1 and 2, in showing that the hysteresis occurs only to a small extent in systems of caseinate dispersed in dialysate, but that it is very prominent in both skimmilk and caseinate systems containing serum proteins.

Effect of β-lactoglobulin on rennet coagulation. To determine whether all or only some of the serum proteins exhibit this behavior, the individual proteins were incorporated separately and in a mixture in caseinate dispersions in approximately the proportions in which they occur in skimmilk. The data in Table 4 reveal that at levels comparable to those in milk only the β-lactoglobulin caused the increased rennet clotting time upon heating.

TABLE 4
Effect of heat treatment and presence of individual serum proteins on rennet clotting time of caseinate dispersions in milk dialysate

Sample		Rennet clotting time (sec.)		
			Heated 85° C. for 30 min.	
		No heating	Immediate	After 24 hr. at 5° C.
1	Skimmilk	35	130	420
2	Casein in dialysate	28	38	70
3	2 + Milk serum protein *(687 mg/100 ml.)*	24	183	310
4	2 + β-Lactoglobulin *(350 mg/100 ml.)*	27	150	330
5	2 + α-Lactalbumin *(100 mg/100 ml.)*	23	30	70
6	2 + Bovine blood serum albumin *(37.5 mg/100 ml.)*	24	30	75
7	2 + Euglobulin *(37.5 mg/100 ml.)*	29	34	86
8	2 + Pseudoglobulin *(37.5 mg/100 ml.)*	24	38	65
9	2 + Proteins as in 4, 5, 6, 7, 8	25	265	360

Beta-lactoglobulin is further implicated in the effects of heat on rennet clotting by an experiment in which 0.2% of β-lactoglobulin was added to milk before heat treatment (Table 5). The added β-lactoglobulin did not change

TABLE 5

Effect of adding 0.2% β-lactoglobulin on rennet coagulability

		Rennet clotting time (sec.)							
		No hold at 5° C. after heating				24 hr. at 5° C. after heating			
		Raw		Heat 85° C. − 30 min.		Raw		Heat 85° C. − 30 min.	
Lot	Time at 35° C.	No β	0.2% β	No β	0.2% β	No β	0.2% β	No β	0.2% β
	(min.)								
21	5	75	65	262	643	104	73	>1,020	>1,020
	180	65	57	430	960	62	55	890	>900
22	5	78	66	158	252	76	46	>900	>900
	180	43	47	366	>900	42	45	614	900
23	5	90	72	199	311	62	....	>900	>900
	180	52	46	356	536	45	....	458	>900

the rennet clotting time of the raw product but greatly enhanced the effect of heat and the hysteresis.

It is of interest to determine whether heat treatment and hysteresis affect the primary (enzymatic) or secondary (coagulation) stage of rennet action. The two stages can be isolated by allowing the first to proceed at low temperature and at intervals withdrawing aliquots for determination of coagulation time at higher temperature. In this way, Pyne (15) showed that the primary action of rennet is completed at 16°C. in about the same length of time in freshly heated skimmilk as in skimmilk held at room temperature for 4 hr. after heating. He concluded, therefore, that the hysteresis affects the second or coagulation stage rather than the primary enzymatic action of rennet. An attempt was made to corroborate Pyne's result and to compare the rates of the primary action on raw and heated milks. Each such experiment involved A. raw skimmilk, B. skimmilk freshly heated at 85° C. for 30 min. and quickly cooled to 16° C., and C. skimmilk heated at 85° C. for 30 min., quickly cooled to 16° C., and held for 3 hr. Rennet was added to portions of each lot and at intervals during 1 hr. aliquots were transferred to coagulation tubes and the coagulation time determined at 35° C. Controls consisted of portions of each lot held at 16° C. with no added rennet, aliquots being transferred at intervals to coagulation tubes at 35° C. containing rennet. The final concentration of rennet was, of course, constant in all tubes (0.1 ml. of a 1:20 dilution of rennet extract per 1 ml. of milk).

The entire experiment was replicated with four separate lots of skimmilk. Plots of the average data for the four lots are given in Figure 2. Since the control for B exhibited considerable hysteresis during the hour's incubation at 16° C., it was deemed advisable to compare A, B, and C on the basis of the

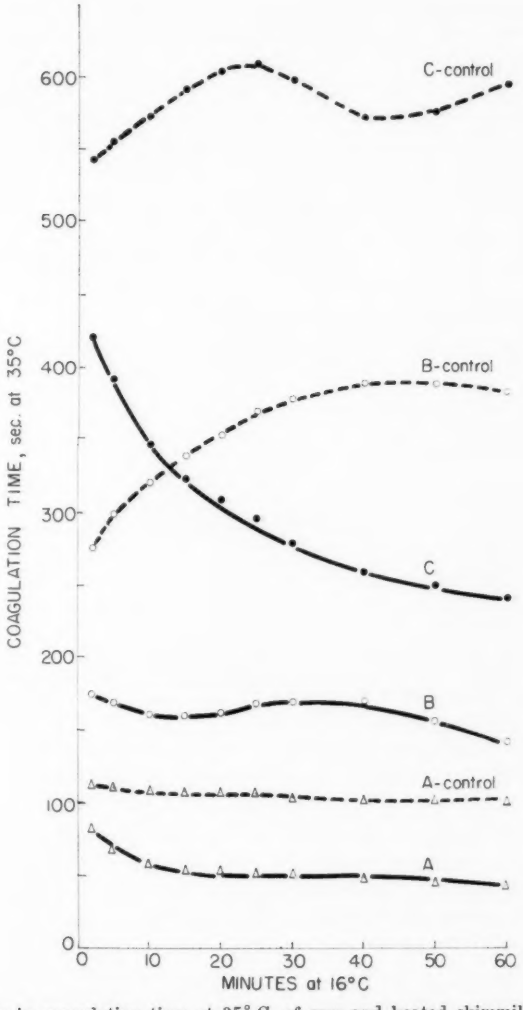


FIG. 2. Change in coagulation time at 35° C. of raw and heated skimmilk as a result of incubation with rennet at 16° C.

A = raw skimmilk.
B = skimmilk heated at 85° C.—30 min. and cooled quickly to 16° C.
C = like B but held 3 hr. at 16° C. before adding rennet.
Controls were held at 16° C. without rennet, and transferred to tubes containing rennet at 35° C.

Average of four replicate experiments.

difference between coagulation times of the renneted samples and the controls. Such a comparison based on the average of all four lots is plotted in Figure 3.

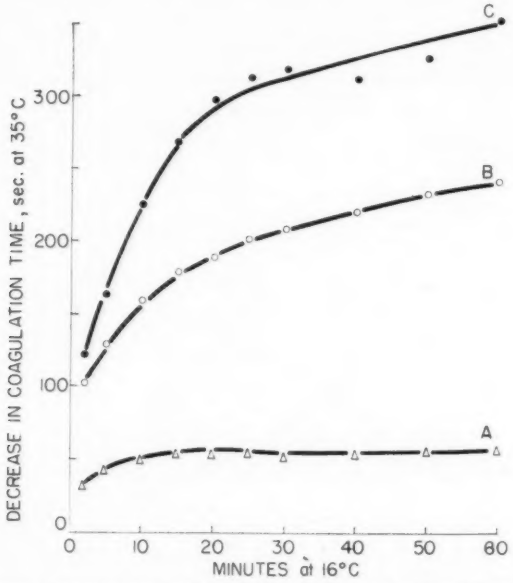


FIG. 3. Difference between coagulation times at 35° C. of skimmilk held at 16° C. with and without rennet.

A = raw skimmilk.
B = skimmilk heated at 85° C.—30 min. and cooled quickly to 16° C.
C = like B but held 3 hr. at 16° C. before adding rennet.

For the raw skimmilk (A) the difference became constant in about 10 min., but for the heated samples (B) and (C) the difference increased throughout the hour's incubation at 16° C. (although at a slower rate after 20 min.). These data suggest that the effect of heating is to inhibit the primary action of rennet and (in agreement with Pyne) that the hysteresis influences the second or coagulation stage of the process.

State of calcium and phosphate. It was thought that β-lactoglobulin may be so altered by heating that it binds a larger fraction of the calcium in the system. Such an introduction of additional calcium-binding capacity into the system would lead one to expect that upon equilibrating a small amount of heated skimmilk by dialysis versus a large amount of raw skimmilk the calcium content in the heated skimmilk should increase. Eight samples of skimmilk were heated at 85 or 90° C. for 30 min. and each then equilibrated by dialysis against a large volume of raw skimmilk for 48 hr. at 0-5° C. (Table 6). At the end of the dialysis period the total calcium content of the heated skimmilk was consistently about 10 mg. per 100 ml. higher than that of the raw skimmilk.

However, the ultrafilterable calcium contents of the raw and heated samples were virtually identical. Thus, the additional calcium was in the colloidal form. The total and colloidal phosphorus likewise increased when heated milk was dialyzed against raw milk. The data for Lot 34 show that dialysis against raw milk for an additional 48 hr. still further increases the content of colloidal calcium phosphate. Furthermore, the uptake of calcium and phosphate occurs even if the heated skimmilk is held at 5° C. for 48 hr. before dialyzing against raw skimmilk for 48 hr. The dialysis of heated milk against raw milk partially restores the rennet coagulability of the former, as might be expected from the increase in colloidal calcium phosphate (Table 7).

TABLE 6

Effect of storage and dialysis vs. raw skimmilk on total and ultrafilterable calcium and phosphate in heated skimmilk

	Total		Ultrafilterable			
				Heated[a]		
Lot	Raw	Heated[a] and dialyzed[b]	Raw	Immediate	48 hr.	Dialyzed[b]
			[*Calcium (mg/ml)*]			
31	1.21	1.31	0.38	0.33	0.35	0.37
32	1.25	1.29	0.37	0.31	0.32	0.33
33	1.20	1.26	0.38	0.33	0.36	0.35
34a	1.22	1.27	0.37	0.29	0.31	0.33
34b[c]	.....	1.28	....	....	....	0.30
34c[d]	.....	1.34	....	....	....	0.33
35	1.15	1.26	0.37	0.30	0.35	0.36
36	1.12	1.22	0.39	0.33	0.35	0.39
37	1.21	1.31	0.38	0.32	0.35	0.36
38	1.20	1.29	0.38	0.31	0.35	0.37
			[*Phosphorus (mg/ml)*]			
31	0.99	1.02	0.37	0.32	0.35	0.36
32	0.99	1.02	0.38	0.33	0.35	0.36
33	0.98	1.01	0.36	0.33	0.35	0.36
34a	0.98	1.00	0.35	0.31	0.33	0.34
34b[c]	.....	1.01	....	....	....	0.34
34c[d]	.....	1.03	....	....	....	0.35
35	0.99	1.04	0.37	0.32	0.34	0.43
36	1.00	1.06	0.37	0.32	0.32	0.37
37	0.96	1.02	0.36	0.32	0.34	0.36
38	0.98	1.07	0.35	0.34	0.35	0.32

[a] Heated at 85° C. for 30 min.
[b] Dialyzed vs. large volume of raw skimmilk for 48 hr. at 5° C.
[c] 34b held 48 hr. at 5° C. and then dialyzed vs. raw milk 48 hr. at 5° C.
[d] 34c dialyzed vs. raw milk for 96 hr. at 5° C.

The effect of direct addition of calcium before and after heat treatment at 90° C. for 30 min. was next determined. For these experiments 0.01% calcium was added in the form of a dry mixture of calcium chloride and calcium hydroxide to avoid changes in pH. Added calcium was more effective in restoring rennet coagulability when it was added after heat treatment (Table 8). This finding suggests that calcium added before heating is immobilized in such a manner by the heating that it is no longer effective in promoting rennet clotting.

If the change in calcium actually involves the β-lactoglobulin, then the addition of more β-lactoglobulin to milk before heat treatment should enhance the uptake of calcium by heated milk when it is dialyzed against raw milk. Accordingly, 0.2% β-lactoglobulin was added to skimmilk before heating. This additional β-lactoglobulin did not, however, increase the uptake of calcium by heated skimmilk upon dialysis versus raw skimmilk (Table 9). Furthermore, an artificial milk prepared by dispersing centrifuged caseinate in the milk ultrafiltrate exhibited an uptake of calcium of similar magnitude to that of heated skimmilk when heated and dialyzed versus raw skimmilk (Table 9). Likewise, rennet whey when heated exhibited a reduction in ultrafilterable calcium and phosphate to a degree comparable to the reduction in skimmilk (Table 10). However, the calcium did not revert to the dissolved state quite as completely upon storage for 24-28 hr. at 0-5° C. in the heated whey as it did in the heated skimmilk. When heated whey was dialyzed against raw skimmilk, it exhibited the uptake of calcium in the same manner as heated skimmilk dialyzed against raw skimmilk. Thus, it appears that this phenomenon can occur in the absence of either the whey protein or the caseinate particles.

These experiments seem to exclude the hypothesis that β-lactoglobulin is so altered by heat treatment as to bind calcium in addition to what it binds in the native state. β-Lactoglobulin seems to be involved in the rennet coagulation problem, but apparently it does not exert this effect by binding part of the calcium.

DISCUSSION

Artificial systems consisting of caseinate centrifuged from skimmilk and dispersed in milk dialysate offer considerable promise for investigations of the effects of various materials and treatments on the rennet coagulability of milk. Although only about 90% of the caseinate could be sedimented by the technique used, no ingredient essential for normal rennet coagulation appears to have been lost. It is difficult to reconcile the failure of heat treatment to prolong the rennet coagulation of these systems with the data of Powell and Palmer (14) and of Pyne (15) showing the occurrence of both the immediate effect and the hysteresis in artificial caseinate-phosphate systems devoid of serum proteins. It is believed, however, that the systems employed in the present work are at least as representative of the actual situation in milk as those employed by previous workers.

The results of this investigation suggest that β-lactoglobulin is involved both in the immediate and in the delayed (hysteresis) prolongation of clotting time by heat treatment. These effects are much less marked with systems low in serum proteins (0.1% or lower) than with those containing a normal level of these proteins. They are further enhanced by addition of β-lactoglobulin to skimmilk. It appears that the β-lactoglobulin must be heated in the presence of the caseinate. Its ability to engage in this reaction is lost if it is previously heated in the absence of casein, as in centrifuge wheys. This fact, as well as the failure of β-lactoglobulin added to skimmilk before heating to enhance the uptake of calcium when heated milk is dialyzed against raw milk, indicates

TABLE 7

Restoration of rennet clotting by dialysis of heated skimmilk vs. raw skimmilk

Lot	Time[a] at 35° C.	Rennet clotting time (sec.) Raw skim		Skim heated 90° C. — 30 min.		
		Fresh	48 hr. at 5° C.	Immed.	48 hr. at 5° C.	Dialyzed vs. raw 48 hr. at 5° C.
	(min.)					
41	5	48	42	90	254	132
42	5	36	38	57	246	113
42	60	36	32	68	122	100
43	60	26	28	113	310	114
44	60	39	46	200	350	158
45	5	47	....	105	.....	.....
45	60	36	35	84	315	196
46	5	35	....	70	.....	.....
46	60	31	30	88	181	141

[a] Time of holding at 35° C. before adding rennet.

TABLE 8

Effect of adding calcium on rennet coagulability of skimmilk

Lot	Time held at 5° C.	Rennet clotting time (sec.)[a] Raw skim		Skim heated 90° C. — 30 min.		
		No Ca	0.01% Ca	No Ca	0.01% Before heat	0.01% After heat
	(hr.)					
43	0	26	20	113	73	56
	48	28	15	310	118	92
44	0	39	33	200	95	65
	48	46	38	350	126	100
45	0	36	28	84	76	....
	48	35	28	315	112	....
46	0	31	20	88	40	....
	48	30	29	181	76	....

[a] All held at 35° C. for 1 hr. before adding rennet.

TABLE 9

Effect of heat treatment on calcium in milk and artificial systems

	Lot 38 Treatment	Calcium content *(mg/ml)* Skimmilk		Skimmilk + 0.2% β-lactoglobulin		Caseinate in dialysate	
		Total	Ultra-filtrate	Total	Ultra-filtrate	Total	Ultra-filtrate
1	Raw	1.20	.38	1.22	.34	1.30	.37
2	Heated 85° C. 30 min.	.....	.31	.....	.31	.....	.31
3	Like 2, but held 48 hr. at 0-5° C.	.....	.35	.....	.34	.....	.34
4	Like 2, but dialyzed vs. raw skim 48 hr.	1.29	.37	1.29	.35	1.40	.37

that β-lactoglobulin does not exert its influence on rennet clotting by binding additional calcium after heat denaturation. Zittle and DellaMonica (19) also found that heat treatment of β-lactoglobulin did not increase its calcium-binding capacity.

TABLE 10

Effect of storage and dialysis vs. raw skimmilk on total and ultrafilterable calcium and phosphate in heated whey

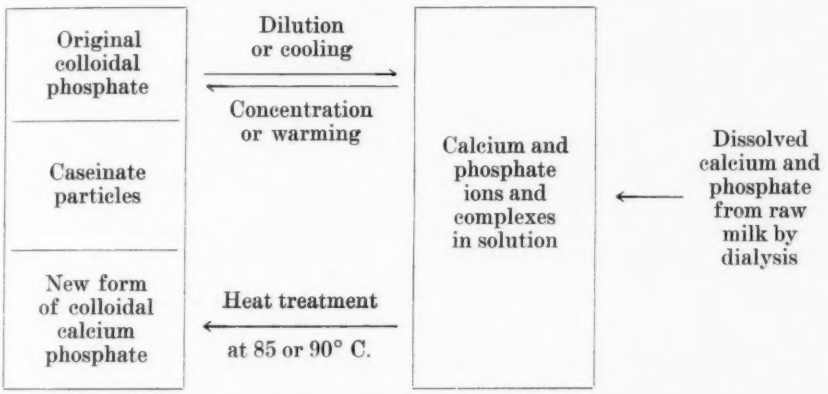
	Total		Ultrafilterable			
				Heated[b]		
Lot[a]	Raw	Heated[b] and dialyzed[c] 48 hr.	Raw	Immediate	48 hr.	Dialyzed[c] 48 hr.
			[*Calcium (mg/ml)*]			
31	0.35	0.50	0.37	0.27	0.31	0.40
32	0.41	0.55	0.34	0.24	0.26	0.31
33	0.40	0.48	0.35	0.29	0.30	0.36
			[*Phosphorus (mg/ml)*]			
31	0.47	0.54	0.36	0.32	0.33	0.37
32	0.50	0.55	0.36	0.31	0.33	0.38
33	0.49	0.50	0.36	0.32	0.34	0.37

[a] Numbers refer to same lots as in Table 6.
[b] Heated at 85° C. for 30 min.
[c] Dinalyzed against a large volume of raw skimmilk for 48 hr. at 5° C.

These experiments suggest that heat treatment changes β-lactoglobulin in such a way that it interferes with the primary action of rennet. One obvious possibility is that β-lactoglobulin combines with one or more of the caseins and hinders the action of rennin. McGugan *et al.* (10) have suggested an interaction between β-lactoglobulin and α-casein on the basis of changes in electrophoretic patterns when β-lactoglobulin and casein are heated together. Such experiments do not, however, demonstrate conclusively that a complex is formed, because the heating of β-lactoglobulin alone increases its mobility to a value close to that of α-casein. DellaMonica *et al.* (2) found that calcium chloride at a level of 6.0 m*M* per liter preferentially precipitates β-lactoglobulin from heated mixtures of casein and β-lactoglobulin. They concluded that interaction does not occur upon heating. However, their data are not sufficiently precise to preclude the interaction of β-lactoglobulin with a small portion or fraction of the casein, particularly a fraction of low phosphorus content.

Unquestionably, heat treatment causes a transfer of calcium and phosphate from the dissolved to the colloidal state. Cold-aging following heating results in a transfer of colloidal calcium and phosphate to the dissolved state. These phenomena must involve more than a simple formation of an insoluble salt at high temperature and resolution on aging after heating, because heated milk dialyzed against raw milk acquires additional colloidal calcium phosphate. The results can perhaps be explained on the basis that heat treatment produces a new form of colloidal calcium phosphate more or less irreversibly at the expense of the dissolved calcium and phosphate, and probably of the original colloidal calcium phosphate, as well. When heated milk is aged, some of the original

colloidal calcium phosphate dissolves to restore the content of dissolved calcium and phosphate to nearly their original levels in the raw milk. If, however, heated milk is dialyzed against raw milk it acquires additional colloidal calcium phosphate to maintain the equilibrium between the colloidal and dissolved states. The following diagram summarizes this hypothesis:


Original colloidal phosphate
Caseinate particles
New form of colloidal calcium phosphate
Dilution or cooling
Concentration or warming
Heat treatment
at 85 or 90° C.
Calcium and phosphate ions and complexes in solution
Dissolved calcium and phosphate from raw milk by dialysis

Unfortunately, no analytical method is yet available which permits a distinction to be made between the original colloidal calcium phosphate and a new form produced by heating.

The data presented in this paper suggest that the postulated new form of calcium phosphate does not involve β-lactoglobulin. They are, however, in accord with the suggestion of Evenhuis and DeVries (3), that heat treatment produces an almost irreversible transformation of calcium and phosphate in milk into hydroxyapatite. Of course, these data offer no proof that hydroxyapatite actually is the form produced. The data for the eight samples reported in Table 6 simply are not sufficiently precise to permit designation of any specific salt on the basis of ratio of changes in concentration of calcium and phosphorus on heating, aging, and dialysis.

It seems logical to conclude that the prolongation of clotting time which occurs during hysteresis results from the decrease in the amount of colloidal calcium phosphate associated with the caseinate particles. It is, however, difficult to understand why the hysteresis occurs only if β-lactoglobulin is present when the system is heated. Possibly, the interaction of β-lactoglobulin with the caseinate micelles makes the coagulability more critically dependent on the concentration of colloidal calcium phosphate.

ACKNOWLEDGMENTS

A report of work done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was supervised by the Eastern Utilization Research and Development Division of the Agricultural Research Service.

The technical assistance of Nettie Furgala and Edna Regehr in certain phases of the work is gratefully acknowledged.

REFERENCES

(1) Berridge, N. J. An Improved Method of Observing the Clotting of Milk Containing Rennin. J. Dairy Research, 19: 328. 1952.

(2) DellaMonica, E. S., Custer, J. H., and Zittle, C. A. Effect of Calcium Chloride and Heat on Solutions of Mixtures of β-Lactoglobulin and Casein. J. Dairy Sci., 41: 465. 1958.

(3) Evenhuis, N., and DeVries, Th. R. The Condition of Calcium Phosphate in Milk. III. Netherlands Milk and Dairy J., 10: 101. 1956.

(4) Gordon, W. G., and Ziegler, J. α-Lactalbumin. Biochemical Preparations, 4: 16. 1955.

(5) Hilgeman, M., and Jenness, R. Observations on the Effect of Heat Treatment upon the Dissolved Calcium and Phosphorus in Skimmilk. J. Dairy Sci., 34: 483. 1951.

(6) Jenness, R. Titration of Calcium and Magnesium in Milk and Milk Fractions with Ethylenediamine Tetraacetate. Anal. Chem., 25: 966. 1953.

(7) Larson, B. L., and Jenness, R. The Reducing Capacity of Milk as Measured by an Iodimetric Titration. J. Dairy Sci., 33: 896. 1950.

(8) Larson, B. L., and Jenness, R. β-Lactoglobulin. Biochemical Preparations, 4. 23. 1955.

(9) Mattick, E. C. V., and Hallett, H. S. The Effect of Heat on Milk. J. Agr. Sci., 19: 452. 1929.

(10) McGugan, W. A., Zehren, Virginia F., Zehren, V. L., and Swanson, A. M. Interaction Between Casein and β-Lactoglobulin on Heating. Science, 120: 435. 1954.

(11) Moir, G. M. The Effect of Heat upon the Rennin Coagulation. I. J. Dairy Research, 2: 68. 1930.

(12) Moir, G. M. The Effect of Heat upon the Rennin Coagulation. II. J. Dairy Research, 3: 80. 1931.

(13) Powell, M. E. Effects of Time and Temperature of Holding Milk Heat-Treated at Various Temperatures upon Its Subsequent Coagulation by Rennet. J. Dairy Sci., 19: 305. 1936.

(14) Powell, M. E., and Palmer, L. S. Behavior of Caseinate Sols in a Study of a Hysteresis-like Phenomenon in the Rennet Coagulation of Heated Milk. J. Dairy Sci., 18: 401. 1935.

(15) Pyne, G. T. Rennet Hysteresis and the Calcium Phosphate of Milk. Biochem. J., 39: 385. 1945.

(16) Smith, E. L. The Immune Proteins of Bovine Colostrum and Plasma. J. Biol. Chem., 164: 345. 1946.

(17) Sumner, J. B. A Method for the Colorimetric Determination of Phosphorus. Science, 100: 412. 1944.

(18) Tessier, H., and Rose, D. Calcium Ion Concentration in Milk. J. Dairy Sci., 41: 351. 1958.

(19) Zittle, C. A., and DellaMonica, E. S. Viscosity and Flocculation of Heated β-Lactoglobulin Solutions: Effect of Calcium Concentration and pH. J. Dairy Sci., 39: 514. 1956.

APPARENT LOCATION OF LIPASE IN CASEIN

J. D. SKEAN AND W. W. OVERCAST
Department of Dairying, Tennessee Agricultural Experiment Station, Knoxville

SUMMARY

The principal lipase in milk is known to be associated with the casein complex. In this study, the casein obtained from H_2S-treated skimmilk separated by centrifugation at 50,000 r.p.m. possessed almost twice the lipase activity of the casein separated by acidification with 10% lactic acid.

Sols of the casein separated by acidification and centrifugation were prepared in a borate buffer of pH 9.0. These sols were subjected to continuous paper electrophoresis which separated the casein complex into three fractions; presumably, the α-, β-, and γ-caseins. The collected effluents of the separated fractions were studied to determine if they were capable of causing breakdown of milk fat by incubating them in a milk fat substrate for 72 hr. Based on the increases in free fat acidities of these substrates, lipolytic activity was demonstrated only by those effluents bearing protein from the most rapidly migrating casein fraction (α-casein).

There is ample experimental evidence that the principal lipolytic activity of normal cow's milk is associated with the casein fraction (1, 6, 10, 19). However, the source of this activity has eluded attempts to locate it more specifically within the casein fraction (19). In studying bacterial esterases, Greuller and Sierra (11) separated lipase and ali-esterase from a cell-free solution of *Pseudomonas aeruginosa* by means of paper electrophoresis. The identification of a zone of lipase on a paper sheet after electrophoretically separating the enzymes of a fungus has been reported (12). Lederer (13) discussed continuous electrophoresis as a promising means of purifying enzymes in macro quantities. Bamann and Tietz (3) used this method in the separation of seed lipase preparations from *Nigella sativa*. Based on these reports, and the fact that the major casein fractions are paper-electrophoretically separable (14, 16, 20), it seemed feasible to attempt to use continuous paper-electrophoresis to separate these fractions in quantity sufficient to permit the assay of the lipolytic activity of each, and thereby gain further insight as to the location of this enzyme.

MATERIALS AND METHODS

Commercially separated, grade A, raw skimmilk obtained from the separator was used in this experiment. A 1-liter quantity was moderately agitated in an ice bath while H_2S was passed through it for 10 min. (18). Casein to be used as a source of lipolytic activity was separated from the skimmilk by two methods:

A. This procedure was carried out in a room controlled at 3 to 5° C. An 800-ml. quantity of H_2S-treated skimmilk under agitation was acidified to pH 4.7 by dropwise addition of 10% lactic acid. The acidified skimmilk was distributed among four 250-ml. bottles and centrifuged 10 min. at 2,000 r.p.m. on a 7-in. diameter head. The sedimented curd, after decantation of the whey, was

Received for publication January 20, 1961.

transferred into 400 ml. of borate buffer in a Waring Blendor and resuspended with slow-speed agitation. The resulting sol was alkalized to pH 9.0 by dropwise addition of 1 *N* NaOH. Approximately 50 min. elapsed between skimmilk acidification and curd resuspension. The casein sol thus prepared is termed Lipase Source A.

B. Three 250-ml. volumes of H_2S-treated skimmilk at 3-5° C. were passed separately into a Sharples Centrifuge fitted with a clarifier ring-dam and centrifuged 12.5 min. at 50,000 r.p.m. The centrifuge ambient temperature was around 28° C. and the whey emitted from the centrifuge at the end of each run was 38 ± 1° C. The deposited casein gel was scraped from the bowl wall and resuspended in 500 ml. of borate buffer in the low-speed blendor. Approximately 2 hr. were required for the entire procedure. The casein sol thus prepared is termed Lipase Source B.

The borate buffer used in preparing the lipase sources and as the electrophoresis electrolyte was prepared at room temperature in 38-liter lots and stored at 3 to 5° C. Each 38-liter lot contained 48.3 g. sodium borate and 3.9 g. boric acid, had a pH of 9.0, and a calculated ionic strength of 0.02.

Nitrogen estimates were made by a micro-Kjeldahl method (2) and converted to protein content by the factor 6.25. These protein estimates were obtained during the preparation of Source A on the skimmilk and the casein sol. During the preparation of Source B protein estimates were obtained on the skimmilk, on centrifuged whey both before and after removal of residual casein, and on the casein sol before and after dialysis.

One hundred milliliters of the casein sol in 0.75-in. diameter seamless cellulose tubing were dialyzed against 20 volumes of borate buffer at 3 to 5° C. for 24 to 30 hr. prior to electrophoresis. Electrophoretic separations were made in a Spinco Model CP Continuous Flow Paper Electrophoresis Cell [1] in conjunction with a Constat Power Supply. Each electrophoretic run followed a general procedure which was found to give satisfactory results. The cell was operated in the 3 to 5° C. room with the power supply remotely operated at room temperature. Cell assembly and general operation were according to pertinent instructions (17). The cell was fitted with two upper curtains having alternately spaced drip tabs. The upper curtains were cut from lower curtains (Spinco part No. 400-235). The curtains were washed with electrolyte and let drain before positioning in the cell. Reservoir electrolyte was maintained at the 8-cm. level. After complete assembly, the cell was run 24 to 30 hr. with no electrical potential applied across the lower curtain. This period permitted equilibration of apparatus temperature, gave sufficient time for adequate balancing of lower curtain electrolyte flow, and further washed possible contaminants from the curtains. Subsequently, casein sol was applied at the lower curtain sample tab, 4 in. from the cathode side. Sol application was from a 3-cm. diameter sample vessel elevated at a feed rate duodial setting of 1.0, delivering 0.3 ml/hr during Trials I and II, and at a setting of 0.5, delivering 0.15 ml/hr during Trials III and IV. A 16- to 20-hr. equilibration period followed initial

[1] Spinco Division, Beckman Instruments, Inc., Belmont, California.

sample application, during which the receding voltage was manually advanced until the electrical field became stabilized at 1,000 ± 20 V. and 23.5 milliamperes. Collection of effluents from the lower curtain drip tabs was begun 3 to 4 hr. later and continued until 60 ml. were obtained from each tab. This required from 26 to 30 hr.

After completion of collection, in order to visualize the protein on the curtain and hence locate it in the collected effluents, the high-voltage circuit was opened and the curtain quickly removed from the cell, tautly suspended horizontally over a shallow pan, and dried 30 to 40 min. in a 120-130° C. preheated oven. The dried curtain was immersed 1 hr. in 1 liter of 0.1% methanolic bromphenol blue (Spinco B-4 Dye), followed by a 10-min. immersion in each of four 1-liter volumes of 5% acetic acid. Then the curtain was suspended vertically, allowed to drain, and dried 15 min. in the oven. The color of the dye-binding protein on the curtain was intensified by 15 min. of exposure to an ammoniacal atmosphere.

Assays were made of the lipolytic activity of the original skimmilks, casein sols A and B, whey from Source B preparation, and protein-bearing electrophoresis effluents. Increases in free fat acidity (FFA) were determined by the method of Frankel and Tarassuk (7) in a 38% milk fat substrate prepared from heavy cream, as described by these authors. The assay procedure follows: Six g. of cream were weighed into a clean, dry 50-ml. homogenizer flask followed by the addition of 50 ml. of the sample to be assayed. Four grams of skimmilk powder (Super Starlac) were added to the flask just prior to addition of sample material in the case of centrifuge whey and electrophoresis effluents. This assay mixture was agitated 3 min. with a VirTis "45" Macro Homogenizer [2] operated at maximum r.p.m. The homogenizer shaft assembly was immersed in boiling water 1 min. and dried between agitations. Assay mixtures containing skimmilk, casein sol, or centrifuge whey were aged 18 hr. ± 5 min., and those with electrophoresis effluents 72 hr. ± 5 min. Aging was in tightly closed, 6-oz. prescription bottles with intermittent agitation. After aging, the assay mixtures were agitated 2.5 min. in the homogenizer at maximum r.p.m. During all the agitations the assay mixture temperature rose to 38 ± 1° C. Within 1 min. after this temperature was reached, duplicate determinations for the FFA of the assay mixture were begun.

A standard plate count (SPC) and psychrophilic plate count (PPC) were made on each assay mixture on the day aging ended.

RESULTS AND DISCUSSION

Acid-coagulated casein is not considered a good source of milk lipase for experimental work (8, 9, 10, 19), although it has been reported to have lipase and esterase activity comparable to that of the original skimmilk (1). Based on increases in FFA per unit total protein, the ratios of original skimmilk activity to that of Source A and Source B were 1:0.6 and 1:1.1, respectively. These relative lipolytic activities demonstrated by Lipase Sources A and B and

[2] VirTis Company, Gardiner, New York.

TABLE 1

Per cent total protein in skimmilk and derived materials and increases in free fatty acidity (FFA) produced by the skimmilk and derived materials

Material	Trial											
	I			II			III			IV		
	Protein	FFA	FFA[a]	Protein	FFA	FFA[a]	Protein	FFA	FFA[a]	Protein	FFA	FFA[a]
	Av. of duplicates (%)											
Skimmilk	3.35	15.2	100.0	3.44	17.6	100.0	3.5	10.7	100.0	3.69	13.4	100.0
Lipase Source A	4.0	10.6	70.0	4.63	15.5	87.4						
Lipase Source B							3.06	10.4	97.5	3.0	11.8	88.1
Lipase Source B (dialyzed)							2.81			2.94		
Whey							1.37	4.1	38.1	1.69	5.8	43.2
Whey less residual casein							0.87			0.88		

[a] Expressed as per cent of increase produced by original skimmilk.

their original skimmilks were approximated from data in Table 1. Source B demonstrated nearly twice the activity as that of Source A. Tarassuk and Frankel (19) experimented with casein obtained in the same manner as that of Source B, and stated that it would constitute suitable starting material for purification work with milk lipase.

A typical curtain pattern (Trial IV) obtained during the separation of casein fractions by continuous electrophoresis is shown in Figure 1. Separation occurred into three fractions: 1, an ill-defined area of low-mobility components and 2 and 3, which were distinct, faster-migrating bands of protein. Fractions 1, 2, and 3 of this study were considered analogous to the gamma-, beta-, and alpha-casein fractions, respectively, obtained by macro-batch electrophoretic separation of casein on paper strips (14, 16, 20). Distinct resolution of the slower-migrating components was not obtained. This may have been due partially to an insufficient concentration of these components to permit their expression as a distinct band. However, the effluents collected from this area carried protein electrophoretically different from that in the faster-migrating fractions, as contamination or loss of resolution by tailing of faster-migrating components does not occur in continuous electrophoretic separations (4). Even though the background electrolyte flow in the electrophoretic curtain was carefully balanced for each trial, the curtain protein pattern, with respect to band width and position, was peculiar to each trial. The curtain drip tabs from which the effluents bearing the separated fractions were collected during each trial are given in Table 2.

TABLE 2

Effluent drip tabs bearing separated fractions

Trial	Fraction		
	1	2	3
	(Drip tab No.)		
I	(3–6)	(7–12)	(13–22)
II	(3–6)	(7–14)	(15–24)
III	(4–6)	(7–10)	(11–16)
IV	(3–6)	(7–12)	(13–22)

The pre- and post-aging agitations of the assay mixtures gave uniform milk fat dispersion prior to FFA determinations. The pre-aging agitation provided rapid resuspension of the skimmilk powder which was added to those assay mixtures of centrifuge whey or electrophoresis effluents which were low in milk solids. The addition of skimmilk powder to more nearly simulate a normal milk milieu aided materially in maintaining adequate dispersion of the milk fat. Also, the pre-aging agitation combined with its resulting temperature rise served as a lipase activation treatment. After the post-aging agitations, it usually could be predicted which assay mixtures would show an increase in FFA upon actual determination, since these mixtures produced comparatively little or no foam.

FIG. 1. Typical pattern obtained during continuous paper electrophoretic separation of the casein complex.

TABLE 3

Standard plate counts (SPC) and psychrophilic plate counts (PPC) on electrophoresis effluent lipase assay mixtures on the last day of aging at 3 to 5° C.

Assay mixture of effluent No.	Trial I Source A SPC	Trial I Source A PPC	Trial II Source A SPC	Trial II Source A PPC	Trial III Source B SPC	Trial III Source B PPC	Trial IV Source B SPC	Trial IV Source B PPC
(Drip tab No.)								
1 } 2 }	18T	13T	27T	51T				
3 } 4 }	13T	20T	17T	76T			0.4T	120T
5 } 6 }	230T	56T	300T	20T	7.5T	64 T	18 T 0.8T	250T 240T
7 } 8 }	11T	56T	11T	200T	60 T 690 T	97 T 760 T	0.5T 0.3T	550T 290T
9 } 10 }	28T	42T	24T	400T	21 T 140 T	46 T 690 T	0.3T 1.2T	320T 200T
11 } 12 }	70T	28T	15T	53T	27 T 16 T	2.2T 15 T	0.3T 0.4T	300T 600T
13 } 14 }	1.1M	24T	56T	4T	170 T 13 T	9.4T 2.8T	1.3T 0.9T	330T 170T
15 } 16 }	13T	200T	300T	24T	13 T 140 T	0.3T 7.5T	8.1T 0.5T	370T 72T
17 } 18 }	13T	33T	700T	19T	30 T 3 T	1.1T 7.5T	0.4T 0.2T	140T 78T
19 } 20 }	63T	63T	25T	42T	10 T 2 T	100 T 190 T	1.3T 10 T	180T 360T
21 } 22 }	300T	53T	12T	91T	120 T	12 T	1.5T	140T
23 } 24 }	26T	77T	19T	250T				
25 } 26 }	1.1M	63T	49T	15T				
27 } 28 }	42T	25T	420T	300T				
29 } 30 }	48T	84T	12T	21T				
31 } 32 }	600T	600T	220T	8T				

T = Thousands.
M = Millions.

Frankel and Tarassuk (8) present optimum conditions of pH, enzyme and substrate concentration, and reaction time and temperature for the hydrolysis of milk fat in assaying milk lipase activity. The nature of the electrophoresis effluents precluded the establishment of these optima in assaying for lipolytic activity. Moreover, since these optima were ascertained using fresh skimmilk

TABLE 4

Increases in free fat acidity effected by continuous paper electrophoresis effluents during 72 hr. ± 5 min. aging at 3 to 5° C.

Electrophoresis effluent drip tab No.	Trial			
	I[a] Source A	II[a] Source B	III[a] Source B	IV[a] Source B
1, 2	0.0	0.0		
3, 4	0.0	0.0		0.0
5	0.0	0.0	0.0	0.0
6				0.0
7	0.0	0.0	0.0	0.0
8			0.0	0.0
9	0.0	0.0	0.0	0.0
10			0.0	0.0
11	0.0	0.0	1.2	0.0
12			3.1	0.0
13	0.8	0.0	2.4	0.6
14			0.5	1.4
15	1.9	0.8	0.2	1.4
16			0.0	0.7
17	2.3	1.9	0.0	0.0
18			0.0	0.0
19	2.4	2.3	0.0	0.0
20			0.0	0.0
21	0.0	0.6	0 0	0.9
22				
23, 24	0.0	0.0		
25, 26	0.0	0.0		
27, 28	0.0	0.0		
29, 30	0.0	0.0		
31, 32	0.0	0.0		

[a] Average of duplicates to nearest tenth.

or lyophilized skimmilk, i.e., relatively large quantities of enzyme source, they may not be directly applicable to enzyme sources such as the electrophoresis effluents in this study. In earlier trials, attempts were made to concentrate the separate electrophoresis effluents to a protein content comparable to that of milk and then establish the known optimum conditions for the assay. Concentration was done by lyophilization or by evaporation from cellulose tubing in a warm air stream. No lipase activity was detected in any of these preparations,

perhaps due to the deleterious affect of the prolonged time period and further manipulation. In working with the electrophoresis effluents it became apparent that maximum activity was retained with minimum delay and treatment.

The substrate concentration of 4% milk fat was used in conjunction with the extraction-titration method for FFA determination (7). Assay mixtures were aged at 3 to 5° C. to retard bacterial growth. The aging period was extended for the longest practical period of time, to allow sufficient time for the expression of any milk lipase present without encountering bacterial lipolysis in the assay mixture. Considerable bacterial growth did occur (Table 3), but resulted in no detectable lipolysis. The assay mixtures were mechanically agitated throughout the aging period in Trial I, but in subsequent trials were given only infrequent hand agitation. The lessened agitation caused no detected alteration in the lipolytic activity pattern of the electrophoresis effluents.

Increases in FFA produced in 4% milk fat substrate by the electrophoresis effluents are shown in Table 4. In Trials I and II the effluent from each of the 16 double drip tabs was assayed, while in Trials III and IV only those protein-bearing effluents from single drip tabs were assayed. Detectable increases in FFA occurred only in those assay mixtures containing electrophoresis effluents bearing protein from the most rapidly migrating fraction, which is Fraction 3 in Figure 1. Patton (15) has suggested that milk lipase activity may be inherent in casein per se rather than in some minor contaminant. If the lipolytic activity dealt with in this study was an enzymatic expression of the casein per se, then the question arises as to the reason it apparently was restricted to Fraction 3. If Fraction 2 possessed lipolytic activity the available concentration was insufficient to catalyze detectable increases in FFA in this study. The absence of lipolytic activity in Fraction 1, which is analogous to gamma-casein, might be expected, since this fraction is preformed from blood rather than in the udder (5). The currently recognized heterogeneity of the alpha-casein fraction (5), which is analogous to Fraction 3 of this study, enhances the plausibility that it may be the casein fraction which bears lipase, either as a capability of the fraction per se or in the form of some minor, perhaps isolable, constituent.

REFERENCES

(1) Albrecht, T. W., and Jaynes, H. O. Milk Lipase. J. Dairy Sci., 38: 137. 1955.

(2) Association of Official Agricultural Chemists. Official Methods of Analysis. 7th ed. p. 745. Washington, D. C. 1950.

(3) Bamann, E., and Tietz, N. Über das verhalten gereinigter Samenlipase-präparate bei der Elektrophorese. Biochem. Z., 324(7): 502. 1953. (Biol. Abstr., No. 12341. 29: 1222. 1955.)

(4) Block, R. G., Durrum, E. L., and Zweig, G. Paper Chromatography and Paper Electrophoresis. Academic Press Inc., New York. 1955.

(5) Brunner, J. R., Ernstrom, C. A., Hollis, R. A., Larson, B. L., Whitney, R. McL., and Zittle, C. A. Nomenclature of the Proteins of Bovine Milk—First Revision. J. Dairy Sci., 43: 901. 1960.

(6) Dormer, W., and Widmer, H. Homogenization and Milk Rancidity. Milk Plant Monthly, 21(7): 50. 1922.

(7) FRANKEL, E. N., AND TARASSUK, N. P. An Extraction-Titration Method for the Determination of Free Fatty Acids in Rancid Milk and Cream. J. Dairy Sci., 38: 751. 1955.

(8) FRANKEL, E. N., AND TARASSUK, N. P. The Specificity of Milk Lipase. I. Determination of the Lipolytic Activity in Milk Toward Milk Fat and Simpler Esters. J. Dairy Sci., 39: 1506. 1956.

(9) FRANKEL, E. N., AND TARASSUK, N. P. The Specificity of Milk Lipase. III. Differential Inactivation. J. Dairy Sci., 39: 1523. 1956.

(10) GOULD, I. A. Effect of Certain Factors on Lipolysis in Homogenized Raw Milk and Cream. J. Dairy Sci., 25: 869. 1942.

(11) GREULLER, E. H. M., AND SIERRA, G. Studies on Bacterial Esterases. II. Paper-Electrophoresis of the Esterases of *Pseudomonas aeruginosa*. Antoinie Van Leeukenhoek, J. Microbiol. and Serol., 23(3/4): 273. 1957. (Biol. Abstr., No. 23838. 32: 1999. 1958.)

(12) HERRINGTON, B. L. Lipase: A Review. J. Dairy Sci., 37: 755. 1954.

(13) LEDERER, M. An Introduction to Paper Electrophoresis and Related Methods. Elsevier Publ. Co., New York, N. Y. 1957.

(14) MACRAE, H. F., AND BAKER, B. E. Application of Electrophoresis on Paper to the Estimation of Alpha-, Beta-, and Gamma-Casein. J. Dairy Sci., 41: 233. 1958.

(15) PATTON, S. On the Nature of Milk Lipase(s). J. Dairy Sci., 40: 1020. 1957.

(16) SKEAN, J. D., AND OVERCAST, W. W. Changes in the Paper Electrophoretic Protein Patterns of Refrigerated Skimmilk Accompanying the Growth of Three *Pseudomonas* Species. Appl. Microbiol., 8: 335. 1960.

(17) SPINCO DIVISION, BECKMAN INSTRUMENTS, INC. Operating Instructions—Spinco Model CP Continuous Flow Paper Electrophoresis Cell. Belmont, California.

(18) STADHOUDERS, J., AND MULDER, H. Some Observations on Milk Lipase. II. The Destructive Effect of Light on Milk Lipase Activity. Netherlands Milk and Dairy J., 13: 122. 1959.

(19) TARASSUK, N. P., AND FRANKEL, E. N. The Specificity of Milk Lipase. IV. Partition of the Lipase System in Milk. J. Dairy Sci., 40: 418. 1957.

(20) ZWEIG, G., AND BLOCK, R. G. Filter Paper Electrophoresis of α-, β-, and γ-Casein. Federation Proc., 12: 296. 1953.

CHARACTERISTICS OF COMMERCIAL NONFAT DRY MILK FOR COTTAGE CHEESE [1]

H. E. RANDOLPH AND T. KRISTOFFERSEN
Department of Dairy Technology, The Ohio State University
AND
The Ohio Agricultural Experiment Station
Columbus

SUMMARY

Thirty samples of commercial low-heat nonfat dry milk were subjected to bacteriological, chemical, and physical tests to determine their uniformity. Cottage Cheese was made from 17 of the samples to determine the curd-making properties.

Results are presented for standard plate, direct microscopic, psychrophilic, and coliform counts, titratable acidity, lactic acid, ash, calcium, nitrogen, whey protein nitrogen, dispersibility, and rennet curd tension. Powders obtained from different plants varied considerably, whereas those received from the same plant over a 4-mo. period were relatively uniform. About 90% of the samples contained sweet-curdling organisms which were resistant to 80°C. for 10 min.

Considerable differences were observed in the Cottage Cheese-making properties of the reconstituted milks. There was no apparent relationship between curd quality and cooking time, or between any of the bacteriological, chemical, and physical properties and the curd-making characteristics of the powders.

Cutting the curd at lower than normal titratable whey acidities resulted in a marked reduction in the time required for cooking the cheese. This was done without any impairment to curd quality of nonfat dry milks containing relatively low whey protein nitrogen, and low rennet curd tension values.

At frequent but irregular intervals, difficulties are encountered in the manufacture of Cottage Cheese from low-heat nonfat dry milk which results in prolonged cooking periods and in curd of inferior quality (7, 17, 20, 21, 23, 24). The persistence of such difficulties during the years that low-heat nonfat powders have been proposed as the sole source of solids for Cottage Cheese undoubtedly has contributed to a decrease in the popularity of such powders for this purpose (20). Various attempts (7, 8) have been made to develop a simple and rapid test to predetermine accurately the suitability of powder for Cottage Cheese. However, application of such tests to correlate processing treatments with curd-making characteristics has not always succeeded. Very little attention has been given to the bacteriological quality or the chemical composition of a powder as related to its curd-making properties. Either one or both of these characteristics could play an important role.

The purposes of the present investigation were: (a) to obtain detailed information concerning the bacteriological, chemical, and physical characteristics of commercial nonfat milk powders supplied for Cottage Cheese manu-

Received for publication September 24, 1960.

[1] Technical article 16: 60. The Department of Dairy Technology. Journal article 75-60, The Ohio Agricultural Experiment Station. Supported in part by Hatch Funds through the Ohio Agricultural Experiment Station and by the Ohio Dairy Products Research Fund.

facture; (b) to relate these characteristics to the curd-making properties of the reconstituted milks; and (c) to determine the effect of cutting acidity on the curd-making properties of such milks.

EXPERIMENTAL PPROCEDURES

Selection of powders. The study involved two phases: (a) the examination of powders from different plants and (b) several powders from individual plants. For the first phase, one 50- or 100-lb. sample of low-heat nonfat powder was obtained from each of 18 commercial plants selected arbitrarily from nine of the leading nonfat dry milk producing states (22). These included five plants in Wisconsin, three in Ohio, three in Minnesota, two in Iowa, and one each in Idaho, Michigan, Missouri, New York, and Pennsylvania. For the second phase, three additional samples were obtained at monthly intervals from four of these plants located in different states (Wisconsin, Ohio, Minnesota, and Iowa). The powders were stored at 50° F. during the investigation (6 mo.).

Bacteriological quality tests. The powders were examined for total bacterial count, coliform type organisms, psychrophilic bacteria, and direct microscopic count according to standard methods (5). Levowitz and Weber's (14) single-solution stain was employed for the direct microscopic count. Titratable acidity was determined on 10% reconstituted milk with N/10 NaOH and phenolphthalein indicator. Lactic acid measurements were made according to the Ling method (15) as modified by Cope and Choi (6).

The starter activity test of Horrall and Elliker was employed for detection of inhibitory substances (13). The rate of acid development of a fresh commercial starter in reconstituted milks of the commercial powders was compared with the activity of the starter in a sterile reconstituted milk known to be free of inhibitory substances. The presence of sweet-curdling microorganisms was detected by incubating 9 ml. of milk containing 10% solids (reconstituted in sterile water) for up to 24 hr. at 30° C. Observations for coagulation were made at 2-hr. intervals. Following coagulation, the milks were titrated with N/10 NaOH, with the titer serving as an arbitrary criterion for sweet curdling (coagulation at $< 0.50\%$ acid). The coagulated samples were examined microscopically to determine the morphological characteristics of the microorganisms.

Chemical and physical methods. Ash was determined according to official procedures (4). Calcium was determined according to Reisfeld's modification of the Murexide method (19), and total nitrogen according to the Parnas method (18). The protein content was calculated from the nitrogen values, using the conversion factor of 6.38. Undenatured whey protein nitrogen was measured according to a modified Harland and Ashworth method (3). The relative dispersibility of the powders was determined by the centrifugal method of Gould and Bassett (11), which indicates both the wettability and the solubility of a milk powder.

Rennet curd tension was determined by the method of Emmons *et al.* (8), except that a submarine signal curd Tension Meter (Model 1935) was used. Acid-rennet curd tension values were obtained by a procedure simulating

Cottage Cheese-making conditions. The powders were reconstituted to 11.0% total solids in sterile water. Six hundred milliliters of reconstitute was prepared from each powder and divided equally between three 250-ml. beakers. The milk was adjusted to 90° F. and inoculated with 5% lactic starter. Following incubation at 90° F. for 1.5 hr., rennet was added at the rate of 1 ml. per 1,000 lb. of milk. Incubation was then continued until coagulation occurred. Curd tension measurements were made at 15, 45, and 75 min. following visible coagulation.

Cottage Cheese-making. Cottage Cheese was made in six 55-lb. capacity stainless steel vats constructed to provide optimum control and uniformity. The powders were reconstituted to 11.0% solids and the conventional short-set procedure as outlined by Remaley (20) was followed. Cooking of the curd was controlled with the Lundstedt curd meter (16). A reading of from nine to 11 was considered to indicate sufficiently firm curd. No cheese was cooked for more than 3 hr., as cooking beyond this time was considered commercially unacceptable. The finished curd was evaluated, according to A.D.S.A. standards (4), for body and texture and appearance by a panel consisting of three competent judges.

RESULTS AND DISCUSSION

Bacteriological and general quality characteristics. Summary results for the 30 samples are presented in Table 1. Bacteriologically, the majority of the

TABLE 1

Bacteriological properties of commercial low-heat nonfat powders

	Range	Average
Standard plate count[a]	15T.–75T.	39T.
Direct microscopic count[b]	28M.–58M.	41M.
Coliform count/milliliter	<1–2	<1
Psychrophilic count/milliliter	<1–6	<1
Titratable acidity *(%)*	0.13–0.15	0.142
Lactic acid *(% dry basis)*	0.019–0.098	0.040
Starter activity *(% relative acidity)*	73.9–95.2	87.1
Sweet curdling[c, d]		
A. Hours for coagulation	12–20	15
B. Acidity at coagulation	0.125–0.68	0.26

[a] Thousand/gram.
[b] Million/gram.
[c] Incubation temperature 30° C.
[d] One sample did not coagulate in 24 hr.

samples were of Extra Grade according to American Dry Milk Institute Standards (2). Also, the relatively low titratable acidity and lactic acid values would indicate that milk of good bacteriological quality was used in the manufacture of the powders. The starter activity test revealed the powders to be free from inhibitory substances. The observed variations in ability to support the growth of lactic starters may be accepted as normal due to differences in contents of growth-promoting substances of the milks.

With respect to sweet curdling, the majority of the milks coagulated after 12 to 18 hr. of incubation and only one failed to coagulate after 24 hr. At the

time of coagulation, titratable acidity values ranged from 0.12 to 0.68%. The majority of the samples contained less than 0.50% acid, which would indicate the presence of high numbers of sweet-curdling microorganisms. Only two samples contained sufficient acid (0.58 and 0.68%) to have caused coagulation. Microscopic examinations revealed that large rod-shaped organisms were associated with milks coagulating at acidities below 0.20%, whereas short- and medium-chain cocci predominated when coagulation was at acidities above 0.35%. Mixtures of large rods and short-chain cocci were present in milk coagulating at acidities between 0.20 and 0.35%.

The microscopic examinations indicated that the large rods were spore-forming varieties. This observation was supported by selecting powders containing a predominance of rod-shaped bacteria and heating the reconstituted milks at 80° C. for 10 min., followed by cooling and incubation at 30° C. All but one of these milks coagulated within 24 hr. with little or no acid development and contained large numbers of rod-shaped bacteria.

Chemical and physical characteristics. Summary results for the 30 powders are presented in Table 2. Variations observed in the ash, calcium, and nitrogen

TABLE 2

Chemical and physical properties of commercial low-heat nonfat powders

Characteristics	Range	Average
Ash *(%)*	7.57– 8.25	7.87
Calcium *(%)*	1.21– 1.42	1.30
Nitrogen *(%)*	5.18– 5.82	5.49
Protein *(%)*[a]	33.05–37.13	35.03
Whey protein nitrogen[b]	2.52– 7.05	6.11
Dispersibility		
A. Solubility[c]	0.00–16.63	1.78
B. Wettability[d]	0.00– 8.90	1.73
Rennet curd tension *(g.)*	0.00–84.0	50.0

[a] Nitrogen × 6.38.
[b] Milligrams of whey protein nitrogen per gram of nonfat solids.
[c] Per cent increase in the total solids content of the bottom 25% of the centrifuged sample over the control.
[d] Grams of unstrainable material.

contents of these samples may be explained by the normal compositional differences of milk. The values obtained are in agreement with previously reported results (2, 20). Undenatured whey protein, rennet curd tension, and dispersibility values, which to some degree reflect the intensity of the total heat treatment of the powders, indicated considerable variations in processing conditions.

Nine powders ranging in rennet curd tension values from 6 to 84 g. were selected for acid-rennet curd tension measurements. The results in Table 3, arranged in increasing order of rennet curd tension values, indicate acid-rennet curd tension values ranging from 0 to 22 g., 28 to 105 g., and 71 to 170 g. at the three periods of examination, respectively. The rate of increase in curd tension values with increasing incubation time varied for the different samples. Some milks showed the greatest increase between 15 and 45 min. of incubation (Sam-

ples 3, 4, 6, 8) and others between 45 and 75 min. The acid-rennet curd tension values were not related to titratable acidity of the whey and, except for Sample 1, did not relate directly to the rennet curd tension values.

Uniformity of low-heat nonfat dry milk from individual manufacturers. The results for the powders obtained over a 4-mo. period from four individual plants are summarized in Table 4. These powders were quite uniform with respect to direct microscopic count, coliform count, and titratable acidity within each plant. However, differences in excess of 100% were noted in the standard plate counts of the four powders from a plant. This was of particular significance for Plants 3 and 4, since the number of bacteria per gram for two powders was sufficiently high (68,000 and 75,000) to cause degrading of the samples from Extra Grade to Standard Grade according to American Dry Milk Institute Standards (1). The lactic acid contents of the powders from Plants 1, 2, and 3 varied considerably, whereas those of the powders from Plant 4 were very uniform.

TABLE 3

Relationship of rennet curd tension values to acid-rennet curd tension values in selected samples of commercial nonfat powders

Sample No.	Rennet curd tension	Acid-rennet curd tension					
		15 min.[a]		45 min.[a]		75 min.[a]	
		g.	T.A.[b]	g.	T.A.[b]	g.	T.A.[b]
	(g.)						
1	6	0	.46	28	.49	71	.54
2	44	2	.45	60	.48	149	.55
3	47	12	.49	105	.51	125	.54
4	55	6	.49	66	.52	116	.54
5	57	22	.43	77	.49	165	.54
6	66	11	.46	95	.48	132	.54
7	69	9	.45	66	.50	170	.55
8	78	11	.50	100	.51	132	.53
9	84	10	.46	60	.49	154	.56

[a] Minutes after the first visible coagulation.
[b] Per cent titratable whey acidity.

All powders contained sweet-curdling microorganisms. On a plant basis, small variations were apparent in the coagulation times and the titratable acidities of the reconstituted milks. The acidities of 0.12 to 0.13% observed in the coagulated milks of powders from Plant 2 represented a decrease of 0.01 to 0.02% from the original values.

Powders from individual plants did not vary greatly in ash, calcium, nitrogen, and undenatured whey protein contents. The differences were not greater than what may be expected due to natural variations in the milk supply. Similarly, dispersibility values were fairly uniform from month to month. Significant variations were observed in rennet curd tension values of the powders from three of the plants (Plants 1, 2, and 4).

Cottage Cheese-making properties of commercial low-heat nonfat dry milk. Seventeen powders representing a wide range of whey protein nitrogen and

TABLE 4

Variations in bacteriological, chemical, and physical properties of low-heat nonfat dry milk from individual plants

Characteristics	Plant Number							
	1		2		3		4	
	Range	Av.	Range	Av.	Range	Av.	Range	Av.
Standard plate count [a]	18 T.–49 T.	30 T.	15 T.–41 T.	26 T.	30 T.–68 T.	44 T.	35 T.–75 T.	52 T.
Direct microscopic count [b]	41 M.–53 M.	46 M.	30 M.–43 M.	34 M.	33 M.–54 M.	45 M.	25 M.–46 M.	38 M.
Coliform count	<1	<1	<1	<1	<1	<1	<1	<1
Psychrophilic count	<1	<1	<1	<1	<1	<1	<1	<1
Sweet curdling [c]								
A. Hours for coagulation	14–18	16	12–16	14	14–16	15.5	12–12	12
B. Acidity at coagulation	.14–.23	.19	.12–.13	.13	.14–.24	.18	.14–.32	.195
Titratable acidity *(%)*	.14–.15	.145	.14–.145	.14	.14–.15	.15	.14–.14	.14
Lactic acid *(% dry basis)*	0.036–0.098	0.057	0.024–0.045	0.031	0.019–0.049	0.031	0.029–0.032	0.031
Ash *(%)*	7.71–7.80	7.77	7.87–8.25	8.03	8.01–8.05	8.03	7.72–8.06	7.89
Calcium *(%)*	1.26–1.38	1.34	1.26–1.37	1.33	1.21–1.28	1.25	1.26–1.33	1.30
Nitrogen *(%)*	5.53–5.60	5.55	5.26–5.82	5.51	5.18–5.58	5.33	5.48–5.59	5.56
Protein *(%)* [d]	35.26–35.72	35.41	33.56–37.13	35.15	33.30–35.60	34.01	34.96–36.55	35.47
Whey protein nitrogen [e]	6.51–7.06	6.75	5.66–6.32	6.02	5.85–6.32	5.98	6.07–6.56	6.26
Dispersibility								
A. Solubility [f]	0.38–1.76	1.19	0.18–1.75	0.86	0.38–4.10	2.03	0.18–3.26	1.03
B. Wettability [g]	0.57–1.94	1.17	0.65–2.33	1.41	1.98–3.40	2.39	0.19–1.68	0.75
Rennet curd tension *(g.)*	56–78	63	44–74	53	44–47	46	47–55	52

[a] Thousand/gram.
[b] Million/gram.
[c] Incubation temperature 30° C.
[d] Nitrogen × 6.38.
[e] Milligrams of whey protein nitrogen per gram of nonfat solids.
[f] Per cent increase in the total solids content of the bottom 25% of the centrifuged sample over the control.
[g] Grams of unstrainable material.

rennet curd tension values were selected for Cottage Cheese-making. The results in Table 5 are arranged in decreasing order of whey protein nitrogen values.

The cooking times required for the various curds, which included come-up time to 120° F. (75 min.) and subsequent holding at this temperature, ranged from 80 min. to 3 hr., with 14 of the samples requiring 2 hr. or longer. The body and texture scores of the curds ranged from 34.5 to 31.0 (Excellent to Poor). Twelve of the samples received scores of 32.5 or better (Excellent to Good). The most common defects were grainy and mealy. Scores for appearance ranged from 14.5 to 12.5 (Excellent to Fair), with the majority of the samples ranging from 14 to 13 (Good). Most of the curds were criticized for lacking uniformity. There was no apparent relationship between cooking time and curd quality.

Comparisons between the curd-making properties of the powders and the various bacteriological, chemical, and physical characteristics revealed a lack of definite relationships. General relationships were observed between whey protein and rennet curd tension values, and cooking time and curd quality. Powders with relatively high values for whey protein nitrogen and rennet curd tension usually required a shorter cooking time and resulted in better quality curd than powders with low whey protein nitrogen and rennet curd tension values. However, marked exceptions to this general trend were noted. For example, Powder 7 (Table 5) contained relatively high whey protein nitrogen and rennet curd tension values, but yielded a curd which required an excessively long cooking time (3 hr.). On the other hand, Powder 15 contained relatively low whey protein nitrogen and rennet curd tension values and resulted in a curd which firmed comparatively rapidly (2 hr.).

Effect of cutting acidity on curd-making properties of low-heat nonfat powders. With regular skimmilk, cutting the coagulum at a low whey acidity (0.40 to 0.45%) results in curd which firms quickly during cooking but which is of a tough, firm character. Since a major criticism of Cottage Cheese made from much commercial low-heat powder is the excessively long period required for firming the curd, an experiment was designed to study the effect of whey acidity at cutting on the resulting curd quality. Powders with relatively low and high whey protein nitrogen and rennet curd tension values were selected for this purpose. The cheese was manufactured according to the standard procedure, except that the curd was cut at titratable whey acidities ranging from 0.40 to 0.65%.

Typical results in Table 6 for selected powders reveal a direct relationship between the acidity of the whey at cutting and the time required for cooking the curd. With powders of low whey protein nitrogen and rennet curd tension values a decrease in cutting acidity from 0.56 to 0.40% resulted in a reduction of the cooking period from approximately 3 hr. to 1.5 hr. The attainment of a close to normal cooking period by cutting at lower whey acidities was accomplished without a decrease in curd quality.

Curd from powder of high whey protein and rennet curd tension values should be cut at recommended acidities. Although cutting at lower acidity will

TABLE 5

Relationship of whey protein nitrogen and curd tension to body and texture and appearance scores and criticisms of Cottage Cheese made from commercial low-heat nonfat dry milk

Sample No.	Whey protein nitrogen	Rennet curd tension	Cooking time		Body and texture		Appearance	
					Score [a]	Criticism [c]	Score [b]	Criticism [c]
			(hr.)	*(min.)*				
1	7.05	61	2	00	34.0	Sl. rubbery	14.5	No criticism
2	6.99	57	2	30	33.0	Sl. grainy, sl. weak	14.0	Sl. lacks uniformity
3	6.92	37	3	00	34.5	No criticism	13.5	D. lacks uniformity
4	6.75	78	2	00	33.0	Sl. grainy	14.0	Sl. lacks uniformity
5	6.70	69	2	30	32.0	D. grainy	13.0	Sl. shattered curd
6	6.68	66	2	20	34.0	Sl. firm	14.0	Sl. lacks uniformity
7	6.68	58	3	00	33.0	D. firm	14.0	Sl. shattered curd
8	6.56	55	1	30	31.0	S. grainy, sl. firm	12.0	D. shattered curd
9	6.32	47	2	40	32.0	S. weak	14.0	Sl. lacks uniformity
10	6.26	45	1	35	32.5	Sl. grainy, sl. firm	13.0	D. lacks uniformity
11	6.13	54	3	00	33.5	Sl. grainy	13.5	D. lacks uniformity
12	6.02	84	1	20	32.5	Sl. grainy, sl. firm	14.0	Sl. shattered curd
13	5.97	6	3	00	32.5	D. weak, sl. grainy	13.0	Shattered curd
14	5.85	74	3	00	33.5	Sl. grainy, sl. weak	13.0	Sl. shattered curd
15	5.85	45	2	00	32.0	D. grainy	13.5	D. lacks uniformity
16	5.84	30	3	00	33.5	Sl. weak	12.5	D. shattered curd
17	5.36	41	3	00	32.0	D. grainy, sl. firm	14.0	Sl. lacks uniformity

[a] Excellent 35–34.5 Good 34–32.5 Fair 32–30 Poor 29.5–0

[b] Excellent 15–14.5 Good 14–13 Fair 12.5–11 Poor 10.5–0

[c] Sl.—slight; D.—distinct; S.—strong.

TABLE 6

Relationship of whey protein nitrogen, curd tension, and cutting acidity to the quality of curd and cooking time of Cottage Cheese made from commercial low-heat nonfat dry milk

Sample No.	Whey protein nitrogen	Rennet curd tension	Cutting acidity	Cooking time		Body and texture			
						Score[a]	Criticism[c]	Score[b]	Criticism[c]
				(hr.)	*(min.)*				
			.65	3	00	33.5	Sl. weak	13.5	Sl. shattered curd
			.56	3	00	34.0	Sl. grainy	14.0	Sl. lacks uniformity
1	5.36	41	.51	2	35	33.5	Sl. grainy, sl. weak	13.5	D. lacks uniformity
			.45	1	45	33.5	Sl. grainy, sl. weak	13.5	D. lacks uniformity
			.40	1	35	34.0	Sl. grainy, sl. firm	13.5	D. lacks uniformity
			.60	3	00	33.5	Sl. weak	13.0	Sl. shattered curd
			.56	3	00	33.0	Sl. grainy	13.0	Sl. shattered curd
2	5.97	6	.50	2	45	32.0	D. weak, sl. grainy	13.5	D. lacks uniformity
			.47	1	55	32.5	D. weak, sl. grainy	13.5	D. lacks uniformity
			.40	1	30	33.5	Sl. grainy, sl. weak	13.5	D. lacks uniformity
			.64	2	20	33.5	Sl. weak	14.0	Sl. lacks uniformity
3	6.02	84	.57	1	25	33.0	Sl. grainy	14.0	Sl. lacks uniformity
			.49	1	15	32.5	Sl. rubbery	13.0	Sl. shattered curd
			.60	2	40	33.5	Sl. grainy	14.0	Sl. lacks uniformity
4	6.75	78	.56	1	45	32.5	Sl. rubbery	14.0	Sl. lacks uniformity
			.52	1	20	32.0	D. rubbery	14.0	Sl. lacks uniformity

[a] Excellent 35–34.5 Good 34–32.5 Fair 32–30 Poor 29.5–0

[b] Excellent 15–14.5 Good 14–13 Fair 12.5–11 Poor 10.5–0

[c] Sl.—slight; D.—distinct; S.—strong.

result in a reduced cooking period, the finished curd will be too firm or tough to be acceptable. Actually, the results with such powders indicated that cutting at slightly higher than normal acidity resulted in finished curds of somewhat better quality than did cutting at the generally recommended acidity.

These results indicate it would be advisable to supplement, or perhaps even supplant, whey protein nitrogen and rennet curd tension measurements with pilot manufacture of Cottage Cheese to assess the suitability of a powder for Cottage Cheese, Stone *et al.* (21) first recommended this approach. However, their objective was to establish if a powder was suitable according to recommended Cottage Cheese manufacturing procedures. These recommendations establish conditions under which a powder has optimum Cottage Cheese curd-making properties. The results obtained in this study would indicate that most, and perhaps all, commercial low-heat nonfat powders are suitable for Cottage Cheese, provided the curd is cut at the proper acidity.

Emmons *et al.* (9, 10) found a relationship between the heat treatment given plain or condensed skimmilk and the whey acidity at cutting of Cottage Cheese curd manufactured from these products. The A-C test was developed as a result of these observations. It is doubtful that irregularities in commercial low-heat nonfat powders for Cottage Cheese are due entirely to their processing heat treatments. However, there is a similarity between the findings of Emmons *et al.* (8) and those of the present study on the relationship of the whey acidity at cutting and the length of the cooking time and the curd quality. This suggests that the A-C test may be suitable for the rapid and simple assessment of the optimum cutting acidity of Cottage Cheese curd manufactured from low-heat nonfat powders.

REFERENCES

(1) American Dairy Science Association. Score Card and Guide for Cottage Cheese. J. Dairy Sci., 41: 214. 1958.

(2) American Dry Milk Institute, Inc. The Grading of Nonfat Dry Milk Solids and Sanitary and Quality Standards. Bull. 911 (rev.). 1954.

(3) American Dry Milk Institute, Inc. The Turbidimetric Estimation of Undenatured Serum Proteins in Nonfat Dry Milk. Unpublished release. 1957.

(4) American Public Health Association. Standard Methods for the Examination of Dairy Products. 10th ed. 1953.

(5) Association of Official Agricultural Chemists. Official Methods of Analysis. 8th ed., p. 267. Washington, D.C. 1955.

(6) Cope, B., and Choi, R. P. Adaptation of the Ling Method for the Determination of Lactic Acid in Nonfat Dry Milk. American Dry Milk Institute, Inc. Unpublished release. 1957.

(7) Coulter, S. T., Jenness, R., and Harland, H. The Manufacture of Low-Heat Nonfat Milk Solids. J. Dairy Sci., 37: 476. 1954.

(8) Emmons, D. B., Merrill, D. W., Swanson, A. M., and Price, W. V. Tests Currently Being Investigated in Research on the Evaluation of Nonfat Dry Milk Solids for Cottage Cheese Manufacture. University of Wisconsin. Unpublished. 1954.

(9) Emmons, D. B., Price, W. V., and Swanson, A. M. The A-C Test. Circ. 541, University of Wisconsin Ext. Service. 7 pp. 1957.

(10) Emmons, D. B., Swanson, A. M., and Price, W. V. Effects of Skimmilk Heat Treatments on Cottage Cheese Manufacture. J. Dairy Sci., 42: 1020. 1959.

(11) GOULD, I. A., AND BASSETT, H. J. Effect of Variations in the Composition of Milk with Special Reference to Fat on the Dispersibility of Dehydrated Milk. Rept. No. 12 for the Quartermaster Corps. 1953.

(12) HARLAND, H. A., AND ASHWORTH, U. S. A Rapid Method for Estimation of Whey Proteins as an Indication of Baking Quality of Nonfat Dry Milk Solids. Food Research, 12: 247. 1947.

(13) HORRALL, B. E., AND ELLIKER, R. P. An Activity Test for Cheddar and Cottage Cheese Starters. J. Dairy Sci., 33: 245. 1950.

(14) LEVOWITZ, D., AND WEBER, M. An Effective "Single Solution Stain." J. Milk and Food Technol., 19(5): 121. 1956.

(15) LING, E. R. The Determination of Lactic Acid in Milk. J. Sci. Agr., 2: 279. 1951.

(16) LUNDSTEDT, E. New Curd Meter Takes Guess Work Out of Cheesemaking. Food Eng., 27(11): 97. 1955.

(17) MORRIS, H. A., COULTER, S. T., COMBS, W. B., AND HEINZEL, L. R. Estimation of the Serum Protein Content as a Method for Evaluating Nonfat Dry Milk Solids for Use in Cottage Cheese Manufacture. J. Dairy Sci., 34: 487. 1951.

(18) PARNAS, J. K. The Kjeldahl Method for Determining Nitrogen by the Method of Parnas and Wagner. Anal. Chem., 114: 261. 1938.

(19) REISFELD, R. A. A Study of the Calcium-Binding Properties of Whole Casein, α-Casein, and β-Casein. Ph.D. thesis, Ohio State University. 1957.

(20) REMALEY, R. J. The Use of Nonfat Dry Milk in Making Cottage Cheese. Milk Ind. Foundation Conv. Proc. Plant Sect. p. 19. 1957.

(21) STONE, W. K., LARGE, P. M., AND GRAF, G. C. Determination of the Curd Making Quality of Nonfat Dry Milk. J. Dairy Sci., 43: 48. 1960.

(22) U. S. DEPT. OF AGR. REPORT. Nonfat Dry Milk Solids Production. 1956. Dairy Products Data Sheet. Olsen Publishing Co., Milwaukee, Wisconsin.

(23) WHITAKER, R. The Selection and Use of Nonfat Dry Milk Solids in the Manufacture of Cottage Cheese. J. Dairy Sci., 39: 231. 1956.

(24) WILKOWSKE, H. H. Relationship Between Titratable Acidity and pH During Lactic Acid Fermentation in Reconstituted Nonfat Milk. J. Dairy Sci., 37: 22. 1954.

EFFECT OF PEPTIDE-RICH EXTRACTS ON ACID PRODUCTION BY OXYTETRACYCLINE- AND PENICILLIN-RESISTANT STARTERS[1]

H. E. KENNEDY[2]
Department of Dairy Technology, Ohio State University, Columbus

SUMMARY

The effect of peptide-rich extracts, including pancreas extract, yeast extract, liver fraction L, tryptone, tryptose, neopeptone, casein hydrolysate, milk protein hydrolysate, and peptonized milk, on the acid production of oxytetracycline-sensitive and -resistant counterparts of *Streptococcus lactis* (10D) in skimmilk was determined. Additions of 0.5% of each stimulant enabled the resistant culture to produce acid in 8 hr., in concentrations equal to or in excess of that of the sensitive culture. The additives did not enable the sensitive culture to produce acid in the presence of the antibiotic.

The effect of pancreas extract on acid production by penicillin-sensitive and -resistant counterparts of five strains of *S. lactis* and two strains of *Streptococcus cremoris* was determined. Six of the seven penicillin-resistant cultures, supplemented with pancreas extract, produced acid in 8 hr. equal to or in excess of that developed by the parent strains. The pancreas extract did not enable the sensitive parent counterparts of these cultures to produce acid in the presence of low levels of penicillin. Likewise, the resistant cultures were unable to develop normal acid after supplementing with pancreas extract, if a level of penicillin was added in excess of the level tolerated by these cultures.

The use of antibiotic-resistant starter cultures to counteract the effect of antibiotic residues in milk generally has not proved successful, because such cultures do not produce acid at a rate or in quantities suitable for manufacturing purposes (2, 4, 5, 8). The decreased acid production by antibiotic-resistant cultures may be a result of specific differences in metabolic activities reported to exist between resistant and sensitive counterparts of lactic acid bacteria (2, 7, 11, 12, 13).

Several investigators have related slow acid production in milk to inability of the starter microorganisms to utilize protein (1, 3, 15). Addition to milk of numerous biological extracts has been reported to overcome this deficiency (1, 3, 6, 9, 15). The stimulatory action of certain of these materials has been attributed to their peptide content (1, 3, 10). Angelotti (2) reported that the addition of casein hydrolysate and yeast extract to milk improved acid production by the oxytetracycline-resistant form of *Streptococcus lactis* (10D).

The purpose of the present study was to investigate the effect of certain peptide-rich extracts on antibiotic-resistant and -sensitive single strain cultures in milk with or without antibiotics added.

Received for publication December 7, 1960.

[1] Article No. 19: 60 of the Department of Dairy Technology, The Ohio State University. Supported by a grant from the U. S. Public Health Service (National Institutes of Health).

[2] Present address: Johnson & Johnson, New Brunswick, N. J.

[3] Trade name—terramycin.

MATERIALS AND METHODS

The cultures used were the oxytetracycline[3]-susceptible and -resistant counterparts of *S. lactis* (10D), and the penicillin-sensitive and -resistant counterparts of *Streptococcus cremoris* (Strains 806 and US3) and *S. lactis* (Strains 924, 10D, C10, F1K, and T13b). These cultures were studied in sterile skimmilk (10% reconstituted of nonfat dry milk) which was free of inhibitory substances (Matrix[4]). Daily transfers were made using 1% inoculum, and incubation was for approximately 16 hr. at 30° C. Penicillin- and oxytetracycline-resistant cultures were carried in the presence of 0.5 unit penicillin/milliliter and 100 μg. oxytetracycline/milliliter, respectively.

The nutritional supplements studied included pancreas extract, liver fraction L, yeast extract, tryptone, tryptose, neopeptone, casein hydrolysate, milk protein hydrolysate, and peptonized milk. These nutrients were added as sterile aqueous solutions in concentrations of 0.1, 0.5, and 1.0% to the skimmilk medium. The antibiotics, oxytetracycline or penicillin, were added as aqueous solutions also. Constant volumes were maintained by the addition of small quantities of sterile distilled water.

Inoculations were made using 5% of fresh 14- to 16-hr.-old cultures and incubation was at 30° C. Titratable acidity determinations were made immediately after inoculation (0 hr.) and after appropriate incubation intervals (4, 8, 12, and 24 hr.). The results were expressed as per cent developed acidity, which consisted of the difference between the original titratable acidity and the acidity at a given incubation time.

RESULTS

Effect of additives on oxytetracycline-sensitive and -resistant counterparts of S. lactis (10D). For the first phase of the study, the effect of pancreas extract on the rate and total acid production by the oxytetracycline-sensitive and -resistant counterparts of *S. lactis* (10D), was determined over a 24-hr. incubation period. The curves in Figure 1 illustrate that the sensitive and resistant cultures were stimulated by pancreas extract; however, the degree of relative stimulation was greater for the resistant form. Maximum stimulation for the sensitive culture was obtained with 0.1% pancreas extract, whereas 0.5% gave highest acidity values for the resistant strain. Comparison of the curves which represent acid production by the resistant culture supplemented with 0.5% pancreas extract and by the sensitive control culture, reveals that the rate of acid production by the resistant culture was stimulated to such an extent that after 8 hr. of incubation it had developed acidity equal to that produced by the sensitive control, i.e., 0.47% developed acidity. This value is appreciably higher than that for the resistant control, which was 0.23%. The pancreas extract had a greater effect on the rate than on total acid production.

For the second phase of the study, the effect of the other peptide-rich extracts on acid production by oxytetracycline-sensitive and -resistant *S. lactis* (10D) within an 8-hr. incubation period was determined. The additives may

[4] Galloway-West Co., Chicago.

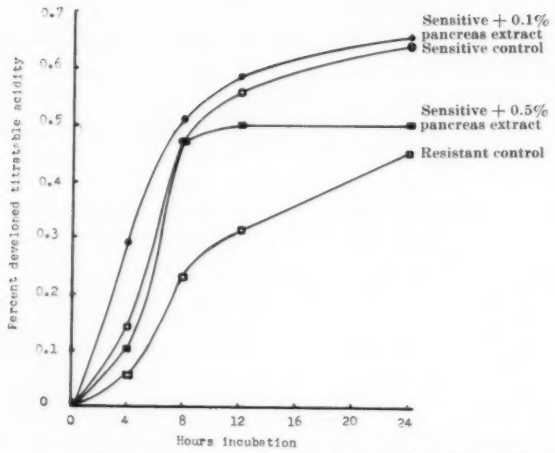


FIG. 1. The effect of pancreas extract on acid production by oxytetracycline-resistant *Streptococcus lactis* (10D).

be grouped generally into three categories, i.e., water-soluble portions of glandular or yeast proteins, peptides obtained by digestion of casein or other proteins, and enzymatic digest of whole milk proteins. Generally, the lowest concentration (0.1%) effected somewhat less stimulation than the higher dosages, although little difference was observed between the results obtained with 0.5 and 1.0% of the additives. Table 1 contains data representative of average developed acidity values obtained with the supplements at the 0.5% concentration. These data reveal differences in acid production by the unsupplemented sensitive and resistant control cultures and the effectiveness of each of the stimulants on acid

TABLE 1

Effect of 0.5% peptide-rich extracts on acid production by oxytetracycline-sensitive and -resistant *Streptococcus lactis* (10D)

	Per cent developed titratable acidity [a]					
	Sensitive			Resistant		
Additive	Control	Additive	Per cent increase	Control	Additive	Per cent increase
Glandular or yeast proteins						
Pancreas extract	.41	.54	32	.22	.48	129
Liver fraction L	.42	.53	28	.23	.45	96
Yeast extract	.42	.54	29	.23	.46	100
Digested casein or other proteins						
Tryptone	.40	.50	25	.26	.48	85
Tryptose	.41	.53	29	.26	.42	62
Neopeptone	.40	.49	23	.26	.46	73
Casein hydrolysate	.39	.51	31	.23	.46	100
Digested whole milk proteins						
Milk protein hydrolysate	.39	.50	28	.24	.53	121
Peptonized milk	.39	.46	18	.24	.40	67

[a] Average values for four trials.

production by both of these culture counterparts. Acid production by the resistant culture supplemented with 0.5% additives reached a level equal to or in excess of that of the sensitive controls. The range of percentage increase over the controls by the resistant culture was from 67 to 129%, whereas the increase by the sensitive culture was only from 18 to 32%.

Effect of additives in the presence of oxytetracycline on acid production by oxytetracycline-sensitive and -resistant counterparts of S. lactis (10D). The effect of the additives on acid production by both the sensitive and resistant cultures in the presence of 5 μg. oxytetracycline/milliliter was investigated. For this purpose, determinations were made in milk supplemented with either 0.5% additive, 5 μg. oxytetracycline/milliliter, or 0.5% additive plus 5 μg. oxytetracycline/milliliter. The data in Table 2 illustrate that acid production

TABLE 2

Effect of 0.5% nutritional supplements in milk on acid production by oxytetracycline-sensitive and -resistant *Streptococcus lactis* (10D) in the presence of 5 μg/ml oxytetracycline

	Per cent developed titratable acidity [a]							
	Sensitive				Resistant			
Additive	Control	Additive	O.T.[b]	Additive + O.T.	Control	Additive	O.T.	Additive + O.T.
Glandular or yeast proteins								
Pancreas extract	.38	.57	.02	.02	.20	.48	.20	.47
Liver fraction L	.38	.51	.03	.05	.21	.43	.21	.46
Yeast extract	.38	.55	.03	.06	.21	.45	.21	.45
Digested casein or other proteins								
Tryptone	.41	.51	.03	.05	.31	.50	.33	.48
Tryptose	.41	.53	.03	.05	.31	.46	.33	.46
Neopeptone	.41	.48	.03	.03	.31	.49	.33	.45
Casein hydrolysate	.38	.53	.03	.06	.21	.46	.21	.44
Digested whole milk proteins								
Milk protein hydrolysate	.41	.50	.03	.05	.31	.62	.33	.53
Peptonized milk	.41	.46	.03	.04	.31	.40	.33	.46

[a] Average values for two trials.
[b] Oxytetracycline (5 μg/ml).

by the sensitive culture was inhibited by oxytetracycline, regardless of the presence of the nutritional additives; whereas, the acidity of the supplemented resistant culture was increased beyond its control and to levels equal to or in excess of the sensitive controls. The results for all additives may be represented by values for pancreas extract, which show that 0.5% of this material increased the acidity of the sensitive culture from 0.38 to 0.57%; however, upon addition of 5 μg. oxytetracycline/milliliter, the developed acidity was only 0.02%. The resistant culture supplemented with pancreas extract had a developed acidity value of 0.48%, compared with its control value of 0.20%. In the presence of pancreas extract and oxytetracycline, the resistant culture produced 0.47% acidity.

Effect of pancreas extract on penicillin-sensitive and -resistant counterparts of S. lactis and S. cremoris. The effect of 0.5% pancreas extract plus 0.5 unit penicillin/milliliter milk on acid production by the penicillin-sensitive and -resistant counterparts (8) of five strains of *S. lactis* and two strains of *S. cremoris* was determined. Similarly, the effect of 0.5% pancreas extract in the presence of 1.0 unit penicillin/milliliter milk on acid production by only the resistant counterpart of each culture was studied. One unit of penicillin/milliliter was a level twice as great as that to which these cultures were adapted. Developed acidity values, measured after 8 and 24 hr. of incubation, are presented in Table 3. The pancreas extract did not counteract the effect of penicillin on the sensitive cultures, but the resistant cultures were appreciably stimulated by pancreas extract in the presence of 0.5 unit penicillin/milliliter. For example, after 8 hr. of incubation, the sensitive culture, *S. lactis* (C10), developed 0.38% acidity; however, this culture supplemented with pancreas extract and penicillin developed only 0.25%. The values after 24 hr. of incubation were 0.60 and 0.30%, respectively. The developed acidity of the resistant *S. lactis* (C10), for example, was only 0.25% after 8 hr., but pancreas extract stimulated the acid production to a value of 0.46%. The resistant cultures (except US_3), supplemented with 0.5% pancreas extract and 0.5 unit penicillin/milliliter, produced acidity equal to or in excess of that produced by their parent sensitive controls within 8 hr. of incubation.

TABLE 3

Effect of pancreas extract on acid production by penicillin-sensitive and -resistant starter cultures

			Per cent developed titratable acidity [a]				
			Sensitive		Resistant		
Cultures	Strain No.	Hours incubation	Control	0.5% p.e.[b] 0.5 U. pen.	Control	0.5% p.e. 0.5 U. pen.	0.5% p.e. 1.0 U. pen.
Streptococcus lactis	(924)	8	.41	.10	.19	.43	.23
		24	.57	.29	.40	.55	.46
	(10D)	8	.11	.07	.16	.46	.16
		24	.36	.37	.44	.53	.44
	(C10)	8	.38	.25	.25	.46	.22
		24	.60	.30	.42	.50	.43
	(FIK)	8	.18	.07	.18	.50	.19
		24	.40	.33	.42	.48	.33
	(T13b)	8	.16	.24	.27	.48	.42
		24	.42	.38	.41	.52	.41
Streptococcus cremoris	(806)	8	.45	.12	.15	.48	.15
		24	.58	.30	.48	.54	.39
	(US_3)	8	.59	.10	.25	.29	.16
		24	.58	.28	.43	.45	.33

[a] Average values for two to three trials.
[b] Pancreas extract (0.5%) and penicillin (0.5 unit or 1.0 unit/milliliter milk).

After 8 hr. the supplemented culture, *S. lactis* (T13b), in the presence of 1.0 unit penicillin/milliliter, produced 0.42% acidity compared to 0.27% for the control (Table 3). After 24 hr. there was no difference between the results obtained in supplemented and control milk. The remaining six cultures were inhibited by 1.0 unit penicillin, regardless of the addition of pancreas extract. Thus, in most cases partial penicillin resistance did not complement pancreas extract in overcoming the inhibitory effect of increased penicillin dosages.

DISCUSSION

The results reported show that, by the addition of peptide-rich extracts, acid production by antibiotic resistant starter cultures may be stimulated to a level equal to that of their sensitive counterparts. Slow acid production by antibiotic-resistant lactic streptococci would appear to be due to an altered capacity of these bacteria to utilize the carbohydrates in milk. Indeed, the effect of antibiotic resistance on carbohydrate utilization by lactic streptococci has been well demonstrated (11). However, the necessity of a suitable nitrogen source for microorganisms to conduct fermentative activities under normal conditions is generally recognized.

The stimulatory properties of various extracts of plant and animal origin are attributed to their content of certain peptides, which are readily available for utilization by bacteria. Furthermore, it has been observed (14) that slower-growing cultures responded to more peptides in various extracts than did faster-growing strains; however, all extracts contained certain common stimulatory factors. Presumably, the same situation exists for the antibiotic-resistant counterparts of sensitive cultures. Previous investigators (2, 3) have suggested that increased acid production by peptide supplements may be a result of enhanced enzyme formation.

All stimulants employed in the present study were partially digested proteins and all of them had similar effects on acid production. These facts suggest that the reduced ability for acid production by oxytetracycline- or penicillin-resistant cultures may be associated with a change in ability to utilize protein, as indicated by a more specific requirement for available nitrogen. That is, the resistant cultures may have developed a dependence on specific nitrogen sources in order to synthesize the necessary materials for carbohydrate utilization. This view appears valid as the resistant forms of the cultures responded to stimulants which were inactive for the antibiotic-sensitive counterparts.

ACKNOWLEDGMENT

The author expresses appreciation to Dr. A. C. Findlay of Charles Pfizer and Company, who provided the antibiotics used in these studies.

REFERENCES

(1) Anderson, A. W., and Elliker, P. R. The Nutritional Requirements of Lactic Streptococci Isolated from Starter Cultures. II. A Stimulatory Factor Required for the Rapid Growth of Some Strains in Reconstituted Nonfat Milk Solids. J. Dairy Sci., 36: 608. 1953.

(2) ANGELOTTI, R. Studies on the Physiological Activities of Terramycin-Resistant and -Susceptible Cultures of Cheese Ripening Strains of *Streptococcus lactis*. Ph.D. thesis, Ohio State University. 1955.

(3) GARVIE, E. I., AND MABBITT, L. A. Acid Production in Milk by Starter Cultures—The Effect of Peptone and Other Stimulatory Substances. J. Dairy Research, 23: 305. 1956.

(4) HARGROVE, R. E., WALTER, H. E., MALKAMES, J. P., JR., AND MASKELL, K. T. The Effect of Penicillin and Streptomycin on Swiss Cheese Starters. J. Dairy Sci., 33: 401. 1950.

(5) KATZNELSON, H., AND HOOD, E. G. Influence of Penicillin and Other Antibiotics on Lactic Streptococci in Starter Cultures Used in Cheddar Cheese Making. J. Dairy Sci., 32: 961. 1949.

(6) KENNEDY, H. E., AND SPECK, M. L. Studies on Corn Steep Liquor in the Nutrition of Certain Lactic Acid Bacteria. J. Dairy Sci., 38: 208. 1955.

(7) MIKOLAJCIK, E. M. Physiology of Oxytetracycline-Resistant and -Sensitive *Streptococcus lactis*. Ph.D. thesis, Ohio State University. 1959.

(8) RICHARDS, R. J., KENNEDY, H. E., AND GOULD, I. A. Antibiotic Resistance and Acid Production Among Starter Cultures Belonging to the Genus *Streptococcus*. J. Milk Food Technol. (In press.)

(9) SANDINE, W. E., HANKIN, L., SPECK, M. L., AND AURAND, L. W. Observations on Bacterial Growth Stimulants Present in Pancreas Tissue. J. Dairy Sci., 38: 597. 1955.

(10) SANDINE, W. E., SPECK, M. L., AND AURAND, L. W. Identification of Constituent Amino Acids in a Peptide Stimulatory for Lactic Acid Bacteria. J. Dairy Sci., 39: 1532. 1956.

(11) SHAHANI, K. M. Carbohydrate and Pyruvate Metabolism of Oxytetracycline-Sensitive and Oxytetracycline-Resistant Organisms. Antibiotics Annual 1956-57, p. 523. Medical Encyclopedia, Inc. 1957.

(12) SHAHANI, K. M. Galactokinase and Galactowaldenase Enzyme Activity of *Streptococcus lactis*. J. Dairy Sci., 43: 852. 1960.

(13) SHAHANI, K. M., AND HARPER, W. J. Differences in the Acid Production and Phosphorus Metabolism of Oxytetracycline-Sensitive and -Resistant Organisms. Appl. Microbiol., 6: 9. 1958.

(14) SPECK, M. L., MCANELLY, J. K., AND WILBUR, JEANNE D. Variability in Response of Lactic Streptococci to Stimulants in Extracts of Pancreas, Liver, and Yeast. J. Dairy Sci., 41: 1. 1958.

(15) WRIGHT, L. D., AND SKEGGS, H. R. The Growth Requirements of Certain Streptococci. J. Bacteriol., 48: 117. 1944.

COMPARISON OF FLAVOR AND SOME CHEMICAL PROPERTIES OF INSTANTIZED AND NONINSTANTIZED NONFAT DRY MILK

J. B. MANN AND J. M. McINTIRE

Carnation Research Laboratories, 8015 Van Nuys Boulevard, Van Nuys, California

SUMMARY

This experimental work was planned to study changes in flavor, color, thiobarbituric acid, and acid ferricyanide optical density values on paired samples of instant and noninstant nonfat dry milks under various storage conditions.

Hedonic scores for instant and noninstant milks were initially 5.6 and 5.7, respectively, and were 4.7 and 4.8 after storage at 120°F. Triangular taste tests results of the paired samples indicated no significant difference between most of the pairs, either before or after storage at both temperatures.

Reflectance values at 520 mμ and 400 mμ wave lengths changed very slightly during storage and were approximately the same for both milks.

The average thiobarbituric acid optical density values for the instant nonfat samples were 0.210 before, and 0.244 after storage at 100°F. The corresponding values for the noninstant nonfat samples were 0.103 before, and 0.161 after storage at 100°F. The average acid ferricyanide optical density values were similar to those obtained with the thiobarbituric acid method.

It was concluded that the instantizing process did not adversely affect the flavor of nonfat dry milk, and that the thiobarbituric acid or acid ferricyanide tests for reducing compounds can not be used as an index of flavor quality of nonfat dry milk.

In recent years the retail sales of instantized nonfat dry milk have overshadowed the sales of regular nonfat dry milk, the reason being the ease of reconstitution of the instant product. There has been a controversy regarding the influence of instantizing on flavor quality of nonfat dry milk. Investigators (1, 3) have stated that instantizing causes a stale-cereal flavor. Parker and McIntire (4) have reported that the instantizing process does not adversely affect the flavor of the nonfat dry milk with respect to consumer use.

There has been little work published on a direct comparison of flavor of instant and noninstant nonfat dry milk during storage. Keeney and Bassette (3) observed higher thiobarbituric acid values (TBA) in instant nonfat dry milk than in regular nonfat dry milk. They postulated that the reducing substances measured by TBA were precursors of the browning reaction and attributed a greater off-flavor in instant nonfat dry milk to these compounds.

An increase in acid ferricyanide reducing value (AFR) was observed when nonfat dry milk was instantized (4).

Since browning precursors have been associated with off-flavor in instant nonfat dry milk, it was thought that color (measured as reflectance) might be correlated with off-flavor. Experience in measuring the reflectance of evaporated milk indicated that the 520 mμ and 400 mμ wave lengths could be used to measure browning.

Received for publication October 19, 1960.

Therefore, experimental work was planned to study changes in flavor, color, TBA, and AFR values during storage of paired samples of instant and noninstant nonfat dry milk.

METHODS

Twenty paired samples of instant and noninstant nonfat dry milk were used during this study. The samples were taken from commercial production runs. Before the samples were placed in storage the moisture content of the samples was equilibrated for seven days at 20% relative humidity at a temperature of 68° F. After equilibration the samples were stored in sealed glass jars. Twenty pairs of samples were stored at 120° F. for 2 wk.; this storage condition was used to accelerate the development of any flavor difference. Ten pairs of samples were stored at 100° F. for 12 wk. Our experience has indicated that this time and temperature is equivalent to storage at room temperature (72° F.) for at least 12 mo. Flavor, color, TBA, and AFR determinations were made initially and at weekly intervals on the samples stored at 120° F. The same determinations were made on the ten pairs of samples stored at 100° F., except that these determinations were made at 4-wk. intervals.

The methods of evaluating the samples were as follows:

Flavor. The flavor differences of the samples were determined by two methods, hedonic rating and triangular taste test.

The hedonic ratings were determined by using a scale of one (extreme dislike) to nine (extreme like). Not more than five pairs of samples were examined in one day. Three of the pairs were examined during the morning and two of the pairs were examined in the afternoon of the same day. The flavor panel consisted of nine to 14 people from the technical staff of the Carnation Company Research Laboratories. The milks were reconstituted at the rate of 10 g. of powder to 100 ml. of water. The milks were sampled at 72° F. in random order. The arithmetic average of the scores was determined on each sample.

All the samples stored at 100° F. and ten of the 20 pairs of samples stored at 120° F. were examined initially and at the end of the storage periods by triangular taste testing. The triangular taste test was used to determine if small differences in flavor existed between each pair of samples. The odd sample for each test was determined randomly and the reconstituted instant and noninstant samples were presented to the panel in random order. The judges were asked to pick the odd sample and also to give a hedonic score for each sample. Probability of picking the odd sample was determined from the tables published by Roessler, Warren, and Guymon (5).

Reflectance. Color change in samples was measured by reflectance. The Beckman Model DU Spectrophotometer with the reflectance attachment was used in making the determinations. The reflectance of the reconstituted samples (10 g. powder to 100 ml. tap water) was determined at 520 mμ and 400 mμ wave lengths. The reference standard was a block of reagent-grade magnesium carbonate. This standard was considered as having a reflectance of 100%. A constant amount of sample was used to insure consistent readings. Results are given as per cent reflectance.

Reducing values. Reducing values of the milk were determined by two methods: thiobarbituric acid method (TBA) and acid ferricyanide reducing method (AFR). The TBA method used was Method B as described by Keeney and Bassette (3), except that the results are given in terms of optical density.

The AFR reducing values were determined by the method described by Crowe, Jenness, and Coulter (2), except that the results are given in terms of optical density.

RESULTS

The results of flavor, color, TBA, and AFR values of the samples stored at 100° F. are given in Table 1. The results of flavor, color, TBA, and AFR values of the samples stored at 120° F. are given in Table 2.

Flavor. During 3 mo. of storage at 100° F. the average hedonic ratings of the instant and noninstant samples were essentially the same and did not change over 0.1 unit. The judges showed no consistent preference for either instant or noninstant samples. The probability of picking the odd sample in the triangular taste test was less than the 95% level, indicating no significant difference in the flavor of the samples both before and after 12 wk. storage at 100° F.

The hedonic flavor results for the samples stored at 120° F. showed there was not a preference for either of the milks. Both types of milks dropped approximately 1.0 unit on the hedonic scale after 2 wk. at 120° F. The difference between the two types of milks did not change over 0.1 unit. The triangular taste test was run on each of the ten pairs of samples before and after storage at 120° F. The results for the initial samples showed that the probability of picking the odd sample was less than the 95% level for all of the ten pairs of samples. After storage at 120° F. for 2 wk., the probability of picking the odd sample for three pairs was 99%, the probability of picking the odd sample for two pairs was 95%, and the probability of picking the odd sample for the five other pairs still was less than the 95% level, indicating only a slight difference in flavor of the two types of milks after storage.

The instant samples had higher hedonic scores in four of the five pairs of samples in which there was a significant difference between the two types of milks.

Reflectance. The results for reflectance show that for both the 520 mμ and 400 mμ wave lengths there was practically no difference (less than 2%) between the instant and noninstant samples. During storage the reflectance decreased 2 to 10% for both types of samples at both storage temperatures. After storage the differences between both types of milks were still less than 2%.

Thiobarbituric acid values and acid ferricyanide reducing values. During the 3 mo. of storage at 100° F. there was a gradual rise of the average TBA values of both types of milks. The AFR reducing values also increased. The instant milks had higher initial values than the noninstant milks, as measured by both tests, and the differences between the two types of milks remained about the same during storage at 100° F. for 12 wk.

The average TBA values for the samples stored at 120° F. showed a gradual increase. The differences remained about the same between the two types of

TABLE 1

Results of flavor and chemical evaluation of ten paired samples of instant and noninstant nonfat dry milk before and after 12 wk. of storage at 100° F.

Test	Type of milk	Before	After
Flavor[a]	Instant	5.6	5.5
	Noninstant	5.6	5.6
Thiobarbituric acid values[b]	Instant	0.210	0.244
	Noninstant	0.103	0.161
Acid ferricyanide values[b]	Instant	0.398	0.567
	Noninstant	0.185	0.371
Reflectance (400 mμ)[c]	Instant	68.7	64.6
	Noninstant	69.7	66.6
Reflectance (520 mμ)[d]	Instant	73.1	69.7
	Noninstant	74.2	70.5

[a] Results are given as the average hedonic rating of ten samples, using a hedonic scale of zero (extreme dislike) to nine (extreme like).
[b] Results are given as the average optical density measurements of ten samples.
[c] Results are given as the average per cent reflectance at 400 mμ wave length of ten samples.
[d] Results are given as the average per cent reflectance at 520 mμ wave length of ten samples.

TABLE 2

Results of flavor and chemical evaluation of 20 paired samples of instant and noninstant nonfat dry milk before and after 2 wk. of storage at 120° F.

Test	Type of milk	Before	After
Flavor[a]	Instant	5.6	4.7
	Noninstant	5.7	4.8
Thiobarbituric acid values[b]	Instant	0.168	0.391
	Noninstant	0.075	0.278
Acid ferricyanide values[b]	Instant	0.434	0.807
	Noninstant	0.218	0.633
Reflectance (400 mμ)[c]	Instant	67.3	58.6
	Noninstant	68.4	61.1
Reflectance (520 mμ)[d]	Instant	71.2	64.5
	Noninstant	72.7	70.6

[a] Results are given as the average hedonic rating of 20 samples, using a hedonic scale of zero (extreme dislike) to nine (extreme like).
[b] Results are given as the average optical density measurements of 20 samples.
[c] Results are given as the average per cent reflectance at 400 mμ wave length of 20 samples.
[d] Results are given as the average per cent reflectance at 520 mμ wave length.

milks during storage at 120° F. The average AFR values for the samples stored at 120° F. increased sharply the 1st wk. of storage, but leveled off and increased gradually during the 2nd wk. of storage. The instant milks had a higher initial value than the noninstant milks and the difference between the two remained about the same during storage at 120° F.

DISCUSSION

The main purpose of this study was to determine if the instantizing process influences the flavor quality of nonfat dry milk in respect to consumer use. The results indicated that the flavor acceptability of paired samples of instantized and noninstantized nonfat milks was the same initially and also at the end of the storage periods. It can be concluded from these results that the instantizing process does not adversely affect the flavor of nonfat dry milk.

The other purpose of this study was to measure changes in reducing values and color of the samples during storage, in an attempt to correlate changes in reducing substances and/or color with flavor changes.

The results show that the reducing values measured by both methods were higher for the instant samples and that the values for both types of milk increased during storage. The hedonic flavor scores of both types of milk were the same initially and remained the same during storage. During storage at 100° F. the values did not change. From these results it can be concluded that the TBA and AFR values can not be used as an index of flavor quality of nonfat dry milk.

The results of the color as measured by reflectance indicated no difference between the instant and noninstant samples initially and after storage. There was not enough change in either color or flavor to conclude if color as measured by reflectance could be correlated with flavor changes in the samples.

REFERENCES

(1) Bassette, R. Identification of Some Flavor Compounds from Stored Instant Non-Fat Dry Milk. Ph.D. thesis, University of Maryland. 1958.

(2) Crowe, L. K., Jenness, R., and Coulter, S. T. The Reducing Capacity of Milk and Milk Products as Measured by a Modified Ferricyanide Method. J. Dairy Sci., 31: 595. 1948.

(3) Keeney, M., and Bassette, R. Detection of Intermediate Compounds in the Early Stages of Browning Reaction in Milk Products. J. Dairy Sci., 42: 945. 1959.

(4) Parker, R. B., and McIntire, J. M. Relationship of Ferricyanide Reducing Capacity to Keeping Quality of Nonfat Dry Milk. J. Dairy Sci., 40: 604. 1957.

(5) Roessler, E. B., Warren, J., and Guymon, J. F. Significance in Triangular Taste Tests. Food Research, 13: 503. 1948.

ACCURACY OF THE PLASTIC BEAD METHOD OF MEASURING SOLIDS-NOT-FAT OF MILK UNDER FIELD CONDITIONS[1, 2]

R. C. LAMB,[3] L. D. McGILLIARD, AND J. R. BRUNNER
Departments of Dairy and Food Science, Michigan State University, East Lansing

SUMMARY

The plastic bead method of measuring solids-not-fat (SNF) content of milk is a practical test for use by DHIA supervisors in the field. This method can be as accurate under field conditions as in the laboratory. The mean differences between duplicate readings in the field, between duplicates in the laboratory, and between duplicate readings in the field and the laboratory were .07, .14, and .16% SNF, respectively. Correlations between duplicate readings, between methods, and between field and laboratory were .86 or greater. Although the lactometer method appears slightly more accurate than the bead method, the difference is so small as to merit little consideration in practical use.

In 1957 two methods which appeared practical for large-scale testing of milk for its content of SNF were reported (3, 9). Both of these methods were based on hydrometry, but differed from older lactometric procedures in that they required a smaller sample of milk and less time to perform. The plastic bead method of Golding (3) also differed, in that the equipment was less fragile and the test was reported to be (1) less sensitive to temperature change.

The accuracy of the Watson-pattern lactometer (9) has been reported for testing herd milk samples and samples from individual cows under laboratory conditions (7, 8) and for testing samples from individual cows under field conditions (6). The accuracy of the Golding plastic bead method (3, 4) has been reported for testing samples from individual cows and herd milk samples under laboratory conditions (1, 4, 5). The present study was undertaken to measure the accuracy of the plastic bead method for testing individual samples of milk under field conditions and to compare the accuracy of the method with that of the lactometer and bead methods performed in the laboratory.

EXPERIMENTAL PROCEDURE

Equipment. The apparatus consisted of a 4-oz. sample bottle fitted with a snap-in plastic grid and containing ten plastic beads, each $\frac{5}{16}$ in. in diameter and a different color. Each bead varied in density by 1 mg. per milliliter at 68° F. and represented a density of one lactometer degree between 68 and 76° F. The density of the set of ten beads ranged from 1.025 to 1.034 (1, 3, 4). A glass stand over a tilting stationary mirror was used to aid in reading the number of beads sinking to the bottom of the bottle.

Received for publication November 10, 1960.

[1] Journal Article No. 2706 from Michigan Agricultural Experiment Station.

[2] These studies were supported in part by the Dairy Cattle Research Branch of the Agricultural Research Service, USDA.

[3] Present address: Department of Dairy Industry, Utah State University, Logan, Utah.

Testing procedure. Eight DHIA supervisors in six Michigan counties were provided with test equipment. Seven of the supervisors conducted the test at home; the other one conducted the test on the farms. The DHIA supervisor obtained a 2-oz. sample of milk from each cow and placed it directly into the sample bottle containing the plastic beads. This provided enough milk to conduct both the SNF test and the Babcock test for fat. The milk was heated to approximately 104° by immersing the sample bottle of milk in water at 115-120° F. The milk was held at 104° for 5 min. to liquefy the fat completely and then was mixed thoroughly by rotary motion. The milk was cooled rapidly to 68° F. by immersing the sample bottle in cold water. The milk was again thoroughly mixed at 68° F. The sample bottles were placed over the mirror, and the number of beads sinking to the bottom was recorded. Occasionally, one bead near the exact density of the milk remained in the middle. When this happened, a slight rotary mixing generally resulted in the bead either rising or sinking. Only the number of beads actually sinking was recorded. The temperature of the milk was recorded immediately following the reading.

Method of comparison. Following a one-half-day training session on the equipment and procedure, the DHIA supervisors tested one herd for additional practice. From the second herd tested, quadruplicate samples of milk were obtained from every fourth cow until a total of six cows had been sampled. Duplicate samples were analyzed in the field by the DHIA supervisor. The DHIA supervisor tested the second of the duplicate samples in a separate group following testing of the original samples, but the identities of the duplicates were changed so as to be unknown to the supervisor. The two other samples were analyzed the following day under laboratory conditions. Analyses in the laboratory were made using both the Golding plastic bead method (3, 4) and the small Watson-pattern lactometer (9). Preservatives were not used. Duplicates were not identified to the technician and were tested in random order. All of the herds sampled in this study were registered Guernseys.

Percentage of SNF was calculated using the formulae presented by Erb *et al.* (1) for the plastic beads and by Watson (9) for the small lactometer. Fat content was measured by the supervisors in the field, using the Babcock method.

RESULTS AND DISCUSSION

The temperature of the milk immediately following reading of the number of beads sinking ranged from 66 to 74° F. Since Golding (3) reported no change in the density of the plastic beads between 65 and 78° F., adjustments were not made for temperature.

Analysis of variance showed differences significant at the level of .01 between four of the supervisors and the laboratory and nonsignificant differences between three of the supervisors and the laboratory. Data from one supervisor were not used, because he failed to obtain the specified number of samples and would not conduct the test according to directions.

Partitioning of the variance into its components gave the results presented in Table 1. Differences between samples accounted for over 60% of the variance

TABLE 1

Components of variance for field-laboratory comparison of plastic beads

		DHIA Supervisors													
		1		2		3		4		5		6		7	
Source	D.F.	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%
Sample	5	.39	61	1.34	63	.74	76	1.52	84	1.46	75	.32	28	1.72	87
Location	1	−.02[a]	0	.44	21	.10	10	−.03[a]	0	.41	21	.64	57	−.02[a]	0
S × L	5	−.02[a]	0	−.06[a]	0	.06	6	−.12[a]	0	0	0	0	0	−.09[a]	0
Within-field	6	.10	16	0	0	0	0	.16	9	.08	4	.04	4	.08	4
Within-lab	6	.15	23	.33	16	.08	8	.13	7	0	0	.13	11	.17	9
Total	23	.64		2.11		.98		1.81		1.95		1.13		1.97	

[a] Negative components were considered zero for computing totals and percentages.

for all but one supervisor. Differences between field and laboratory (locations) accounted for no variation for three of the supervisors and were of practical importance for only one supervisor. Variance attributable to differences between duplicates in the field accounted for 0-16% of the variance, whereas the within-laboratory variance ranged from 0-23%. The within-lab variance was larger than the within-field variance in all but two cases, indicating that the DHIA supervisors were able to duplicate their results at least as well as the technician under laboratory conditions. It is not known whether the one-day difference in age of samples or the transportation of the samples from the field to the laboratory could have had any influence on the lower repeatability of results obtained in the laboratory.

The variances within field and within lab are larger in this study than those reported for the lactometric method (6). This may be due in part to measurements being made to a whole lactometer degree with the plastic bead method but estimated to the nearest one-tenth of a lactometer degree with the lactometer method. Thus, there should be fewer differences in the plastic bead readings, but any errors which occur are magnified several times.

The frequency distribution of differences between duplicate plastic bead readings are presented in Table 2. There were no differences between 73% of the duplicates tested in the field, 51% of the duplicates tested in the laboratory, and 34% of the field-laboratory comparisons. None of the duplicate readings in the field differed by more than one bead, while one set of duplicates in the laboratory differed by two beads. Over 60% of the differences between the average of duplicates in the field and the average of the duplicates in the laboratory were less than one bead, and all were less than two beads. There

TABLE 2

Cumulative distribution of differences between duplicate plastic bead readings

	Field		Laboratory		Field-laboratory	
Differences [a]	No.	%	No.	%	No.	%
0	30	73	21	51	14	34
.5	30	73	21	51	25	61
1.0	41	100	40	97	36	88
1.5			40	97	41	100
2.0			41	100		

[a] Differences measured as number of beads.

were six cases (14%) of a difference of two beads between one of the duplicates in the field and one of the corresponding duplicates in the laboratory. The mean differences between duplicates in the field, between duplicates in the laboratory, and between the average of duplicates in the field and the average of duplicates in the laboratory were 0.27, 0.51, and 0.59, respectively. This is equivalent to differences of .07, .14, and .16 SNF for the three comparisons. With one exception, all cases of field-laboratory differences were due to more beads sinking in the field than in the laboratory, resulting in a higher percentage of SNF in the laboratory tests.

The correlations within tester between plastic bead determinations in the field and in the laboratory, between duplicate measurements in the field, and between duplicate measurements in the laboratory were .86, .91, and .86, respectively. The correlations between SNF percentages calculated from bead determinations in the field and lactometer determinations in the laboratory, between bead determinations in the laboratory and lactometer determinations in the laboratory, and between duplicate lactometer measurements in the laboratory were .94, .95, and .98, respectively.

The laboratory technician had little previous experience with the plastic bead method, but was thoroughly familiar and experienced with the lactometer method. This could account for some of the increase in the magnitude of the correlations when the lactometer determinations are included.

The high correlations between duplicate samples, both in the field and in the laboratory, indicate that duplicate determinations add little information and, therefore, are not necessary to estimate accurately the SNF content of milk. The magnitude of the correlations between field and laboratory suggest that, for all practical purposes, the plastic bead method is as useful under field conditions as the bead or the lactometer methods under laboratory conditions.

The range of differences between supervisors suggests a wide difference in capabilities of various supervisors. The data indicate that the Golding plastic bead method can be used as accurately in the field as in the laboratory, but that whether it is used accurately is dependent upon the human element.

The choice of a test for widespread use in routine testing of milk for content of SNF depends on the accuracy, cost, simplicity, and time required to perform the test. Gaunt (2) presents a summary from five experiment stations, which includes a contribution from the Michigan station by the authors, comparing plastic beads, lactometer, and gravimetric methods of measuring SNF. Correlations between bead and lactometer results were larger than those between either and the gravimetric method. Agreement between lactometer and gravimetric methods was slightly closer than between beads and gravimetric. Erb *et al.* (1) reported finding no difference in comparison of bead and lactometer tests with gravimetric assays. These, along with current data, indicate that although the lactometer method is slightly more accurate than the bead method, the difference is not large enough to merit recognition in practice.

The equipment for the plastic bead method has a lower initial cost than that for the lactometer method because of the need for a thermostatically controlled water bath with the latter method. The cost of maintenance and upkeep of the equipment is also higher for the lactometer method for the same reason. The plastic bead test is slightly simpler to perform due to less sensitivity to temperature change. No comparisons of the speeds with which these two methods can be performed have been reported. Thus, the main practical difference between the plastic bead method and the lactometer method of measuring SNF content of milk is the higher cost of equipment for the latter method.

CONCLUSIONS

The Golding plastic bead test can be used as accurately under field conditions as it can in the laboratory. Although the lactometer method appears slightly more accurate than the bead method to measure SNF, the difference is so small as to receive little consideration in practice. The bead method appears more useful under field conditions because of less cost of equipment and because of simplicity of the test. Duplicate determinations are not necessary to estimate accurately the SNF content of milk.

REFERENCES

(1) Erb, R. E., Manus, L. J., and Ashworth, U. S. Comparative Accuracy of the Plastic Bead Method of Golding and the Lactometer Method of Watson for Routine Determination of Solids-not-fat in Milk. J. Dairy Sci., 43: 607. 1960.

(2) Gaunt, S. N. Symposium: Milk Solids-not-fat. Regional Projects and an Extension Viewpoint. J. Dairy Sci., 43: 1532. 1960.

(3) Golding, N. S. Plastics for Hydrometry. Proc. Am. Dairy Sci. Assoc., West. Div. 1957.

(4) Golding, N. S. A Solids-not-fat Test for Milk Using Density Beads as Hydrometers. J. Dairy Sci., 42: 899. 1959.

(5) Golding, N. S., and Manus, L. J. Density Plastic Sphere Hydrometer for Determining Solids-not-fat on Semi-monthly Composite Samples of Producers' Herd Milk. J. Dairy Sci., 43: 858. 1960.

(6) Lamb, R. C., McGilliard, L. D., and Brunner, J. R. Accuracy of a Lactometric Field Test for Measuring Solids-not-fat Content of Milk. J. Dairy Sci., 43: 491. 1960.

(7) Madden, D. E., and Brunner, J. R. Comparative Accuracy of Small Lactometers for Determining the Total Solids of Individual Cows' Milk. J. Dairy Sci., 41: 783. 1958.

(8) Madden, D. E., Brunner, J. R., and Hedrick, T. I. The Value of a Small Lactometer for Determining Total Solids in Herd Milk. Michigan Agr. Expt. Sta., Quart. Bull., 41: 136. 1958.

(9) Watson, P. D. Determination of the Solids in Milk by a Lactometric Method at 102° F. J. Dairy Sci., 40: 394. 1957.

FURTHER COMPARISONS OF ALFALFA HAY AND ALFALFA SILAGE FOR GROWING DAIRY HEIFERS

J. W. THOMAS,[1] L. A. MOORE, AND J. F. SYKES

Animal Husbandry Research Division, Dairy Cattle Research Branch, Agricultural Research Center, Beltsville, Maryland

SUMMARY

Three different types of trials were performed in which body weight gain, dry matter (D. M.) intake, and their ratio were compared when growing dairy heifers were fed hay or silage.

Alfalfa hay and wilted silage simultaneously harvested from the same fields were fed in a continuous (14 mo.) and a reversal trial (7 mo. each) to dairy heifers from 5 to 19 mo. of age. Growth rates, D.M. intake, and efficiency of gain were lower for the heifers fed silage. After 19 mo. of age, all heifers were fed a normal ration of hay, corn silage, and grain, and the growth rate was increased for most heifers. Body weight at 2 yr. of age for heifers that had been fed hay during the 14-mo. experimental period was much greater than that of heifers that had been fed silage during this period. Body weight at 2 yr. for heifers fed silage for either of the 7-mo. periods was somewhat less than that of hay-fed heifers. These data indicate that silage may provide the major source of feed for dairy heifers for as long as 7 mo., either before or after a year of age, provided more adequate rations are supplied following these intervals without seriously reducing their size at calving time.

Alfalfa harvested simultaneously from the same field as hay, wilted silage (43-46% dry matter), or as direct-cut silage during two different crop years was fed for 2 to 3 mo. to growing dairy heifers when 7 to 19 mo. of age. For each crop year there was little difference in rates of gain or dry matter intake or in efficiency of gain for heifers fed hay or wilted silage (43-46% dry matter). Heifers fed direct-cut silage had lower rates of gain, intake, and efficiency. However, in one crop year the difference between hay and direct-cut silage was small and not significant. It is suggested that the dry matter content of the forage when ensiled, and differences in the resulting fermentation, are major factors in determining the acceptability of silages by heifers and their resulting performance.

Dry matter from hay produced larger gains than an equal amount of dry matter from wilted or D.C. silage when heifers were pair-fed the two forages. This suggests that there may be a difference in the nutritive value of hay and silage dry matter, as well as differences in their acceptability by dairy heifers.

It has been demonstrated that dairy heifers attain normal body weights and development when fed limited amounts of milk and grain along with ad libitum access to good-quality hay (1, 10, 13). The substitution of wilted alfalfa silage for the hay during the entire growing period produced lower rates of growth and nutrient intake in heifers (4, 10, 13). (Reference 13 reviews previous results.) The body weight of heifers fed silage to 2 yr. of age was only 80% of the weight of those fed hay.

Heifer feeding trials of short duration have given conflicting results (3, 8, 13) which have been reviewed (12). It appeared desirable to perform additional short-term studies and attempt to identify factors that might influence the comparative performance of heifers when fed silage or hay.

Received for publication August 6, 1960.

[1] Present address: Dairy Department, Michigan State University, East Lansing, Mich.

The present experiments were conducted to determine the effects of feeding alfalfa silage for limited periods of time during different intervals in the growing period. One trial was also designed to determine the ability of heifers to respond to a more adequate ration after the slow rates of growth observed when silage was fed. Other trials indicated that dry matter (D.M.) or other qualities of silages influenced the intake and performance of heifers receiving silages (2, 7), and more specific information on this appeared desirable.

In previous trials, there were differences in efficiency of utilization of silage or hay, but the significance of these differences was complicated by apparent differences in caloric value of gain in heifers fed hay and silage. Consequently, this aspect of the subject was further explored by equalizing the intake of silage and hay in one trial. In most previous trials in which hay and silage were compared each forage was from a different source. It appeared desirable to obtain information on the comparative performance of heifers fed hay or silage harvested simultaneously from the same fields. A preliminary report on one of those trials was previously prepared (11).

EXPERIMENTAL PROCEDURE

In Trial I, 47 heifer calves of the Holstein and Jersey breed were assigned to one of the four experimental dietary treatment groups when 5 mo. of age. All animals were fed the same up to 5 mo. of age. The sequence of the roughage feeding schedule is indicated in Table 1. The treatment was essentially a com-

TABLE 1

Sequence of roughages fed to the 4 groups in Trial I

Group No.	Roughage fed			
	1-5 mo.	5-12 mo.	12-19 mo.	19-24 mo.
1	Hay and silage (1:1)	Hay	Hay	Hay, corn silage, 2 lb. grain
2	Hay and silage (1:1)	Hay	Silage	Hay, corn silage, 2 lb. grain
3	Hay and silage (1:1)	Silage	Hay	Hay, corn silage, 2 lb. grain
4	Hay and silage (1:1)	Silage	Silage	Hay, corn silage, 2 lb. grain

parison of a continuous vs. a switchover feeding schedule during the 5- to 19-mo. age period. Groups 2 and 3 received silage for a 7-mo. period, but at different ages, whereas Group 4 received silage for a 14-mo. period. From birth to 5 mo. of age and from 19 to 24 mo. of age heifers in all four groups received the same feeds. The alfalfa forage fed during the experimental period (5-19 mo.) was harvested simultaneously from the same field as hay or as wilted silage. The silage averaged 31% D.M. when fed.

The feeding schedule of the young calves was similar to that in previous experiments (1, 10, 11, 12). Each heifer received 370 lb. whole milk during the

first 2 mo. of life. A grain mixture containing 15% protein was offered when each calf was ten days of age and increased up to 3 lb. per day as appetite increased. The amount was reduced to 2 lb. at 6 mo. and eliminated when 8 mo. of age. The silage was offered before ten days of age, and the hay at about 1 mo. of age, after which the calves were fed half hay and half silage (dry matter basis). With this procedure, all calves were accustomed to both forages before the comparison was made at 5 mo. of age.

TABLE 2

Body weights, gains, intake, and efficiency of heifers fed hay or silage (Trial I)

	Group				Group			
	1	2	3	4	1	2	3	4
Age *(mo.)*	Holstein				Jersey			
Body weight *(lb.)*								
5	261^{a}	270^{a}	261^{a}	266^{a}	173^{a}	177^{a}	176^{a}	172^{a}
12	605^{A}	589^{A}	466^{B}	460^{B}	382^{A}	402^{A}	283^{B}	249^{B}
19	922^{a}	776^{bc}	851^{ab}	707^{c}	575^{A}	512^{A}	558^{A}	365^{B}
24	1,223^{a}	1,060^{b}	1,125^{ab}	1,033^{b}	686^{A}	703^{A}	707^{A}	570^{B}
24 *(% Std.)*	109	94	100	92	>84	86	86	70
Body weight gain *(lb/day/calf)*								
0-5	1.16^{a}	1.24^{a}	1.22^{a}	1.21^{a}	0.80^{a}	0.83^{a}	0.80^{a}	0.83^{a}
5-12	1.60^{A}	1.49^{A}	0.95^{B}	0.91^{B}	0.97^{A}	1.04^{A}	0.50^{B}	0.36^{B}
st. dev. (±)	.16	.17	.15	.22	.04	.10	.14	.13
12-19	1.51^{AB}	0.89^{C}	1.84^{A}	1.17^{BC}	0.92^{b}	0.53^{c}	1.31^{a}	0.56^{c}
st. dev. (±)	.22	.24	.22	.36	.11	.38	.16	.24
19-24	1.94^{a}	1.84^{a}	1.77^{a}	1.91^{a}	0.78^{b}	1.23^{a}	0.92^{b}	1.23^{a}
st. dev. (±)	.26	.41	.29	.34	.09	.31	.19	.26
5-24 *(lb. total)*	962	790	864	767	513	526	531	398
Intake *(lb. D.M/calf/day)*								
5-12	12.13^{A}	11.25^{A}	8.65^{B}	8.76^{B}	7.66^{A}	8.19^{A}	5.28^{B}	4.78^{B}
st. dev. (±)	.98	.51	.77	1.26	1.13	.76	.83	.63
12-19	19.08^{A}	13.56^{B}	17.94^{A}	12.63^{B}	12.35^{A}	9.59^{B}	12.51^{A}	6.95^{C}
st. dev. (±)	2.93	1.45	3.22	1.67	1.94	1.82	1.18	1.05
19-24	22.62^{a}	18.11^{b}	19.78^{ab}	18.98^{b}	12.98^{a}	13.82^{a}	13.60^{a}	12.26^{a}
5-24 *(total lb.)*	10,118	8,074	8,691	7,676	6,066	6,034	5,870	4,444
Efficiency *(lb. gain/100 lb. D.M. consumed)*								
5-12	13.18^{A}	13.28^{A}	10.91^{B}	10.26^{B}	12.89^{A}	12.74^{A}	9.36^{B}	7.91^{B}
st. dev. (±)	.68	2.31	1.44	1.44	1.70	1.82	1.94	2.77
12-19	7.95^{bc}	6.47^{c}	10.38^{a}	9.22^{ab}	7.16^{b}	5.49^{b}	10.53^{a}	7.90^{b}
st. dev. (±)	.98	1.16	1.17	2.07	1.14	2.61	1.26	3.27
19-24	8.03^{a}	10.22^{a}	8.98^{a}	10.26^{a}	6.13^{b}	8.98^{a}	6.83^{b}	10.07^{a}
5-24	9.50^{a}	9.78^{a}	9.94^{a}	9.99^{a}	8.46^{a}	8.72^{a}	9.05^{a}	8.95^{a}
No. animals	4	5	6	7	5	6	7	7

Sets of averages with the same superscript form a statistically homogeneous grouping. Capital letters, P = 0.01; lower-case letters, P = 0.05.

During two consecutive years (1957 and 1958) alfalfa from the same field was simultaneously harvested as hay, heavily wilted silage (haylage), or direct-cut (D.C.) silage. In Trial II, a second-cutting alfalfa preserved as hay, haylage, and D.C. silage was fed to three groups of three Jersey heifers each. In Trial II-b, the hay and D.C. silage were fed to three Holstein heifers. In Trial III, first-cutting alfalfa as hay, haylage, and D.C. silage of the 1958 crop

TABLE 3
Performance of heifers fed alfalfa harvested three different ways
(averages and standard deviation)

	Dietary treatment group		
	1	2	3
Forage harvested	Hay	Haylage	D.C. Silage
1957 Crop—Trial II			
% D.M. when ensiled	....	50.6	25.8
% D.M. when fed	90	43.0	28.0
No. of calves in Trial II-a	3	3	3
Days fed forage	73	73	73
Initial weight	434	435	465
Daily gain *(lb/calf)*	$0.88^{a} \pm 0.13$	$0.87^{a} \pm 0.29$	$0.76^{a} \pm 0.27$
Daily D.M. intake *(lb/calf)*	$12.45^{a} \pm 4.2$	$11.86^{a} \pm 4.6$	$10.52^{a} \pm 2.5$
Daily D.M. intake *(lb/cwt)*	$2.26^{a} \pm 0.3$	$2.30^{a} \pm 0.2$	$2.50^{a} \pm 0.3$
Efficiency *(lb. gain/100 lb. D.M.)*	$7.98^{a} \pm 4.1$	$7.73^{a} \pm 2.0$	$7.52^{a} \pm 2.4$
No. calves in Trial II-b	3	0	3
Av. initial weight	648	....	658
Daily gain *(lb/calf)*	$1.51^{a} \pm 0.63$	....	$1.11^{a} \pm 0.18$
Daily D.M. intake *(lb/calf)*	$15.37^{a} \pm 4.4$	....	$15.86^{a} \pm 3.2$
Daily D.M. intake *(lb/cwt)*	$2.32^{a} \pm 0.1$	....	$2.08^{b} \pm 0.2$
Efficiency *(lb. gain/100 lb. D.M.)*	$9.51^{a} \pm 1.8$	....	$7.23^{a} \pm 2.0$
1958 Crop—Trial III			
% D.M. when ensiled	....	44.0	20.5
% D.M. when fed	89	46.0	24.0
No. calves in Trial III	4	4	4
Days fed forage	90	90	90
Initial weight	509	496	517
Daily gain *(lb/calf)*	$0.89^{A} \pm 0.21$	$0.93^{A} \pm 0.27$	$-0.29^{B} \pm 0.37$
Daily D.M. intake *(lb/calf)*	$13.09^{a} \pm 0.26$	$13.00^{a} \pm 2.45$	$8.90^{b} \pm 0.21$
Daily D.M. intake *(lb/cwt)*	$2.39^{A} \pm 0.14$	$2.42^{A} \pm 0.13$	$1.79^{B} \pm 0.05$
Efficiency *(lb. gain/100 lb. D.M.)*	$6.72^{A} \pm 1.41$	$7.28^{A} \pm 2.17$	$-3.35^{B} \pm 4.45$

Composition of forages fed						
Trial No.	II	III	II	III	II	III
Crude protein *(%)*	18.5	18.2	21.1	18.6	20.0	18.8
Crude fiber *(%)*	25.9	30.8	27.7	31.3	27.3	33.2
pH	.....	.....	4.9	4.9	4.9	4.9
NH_3-N-as % of total	.....	.....	9.6	11.3	15.9	20.7
Lactic Acid *(%)*	.....	.....	1.6	2.2	2.3	0.9
Acetic Acid *(%)*	.....	.....	1.2	1.5	5.6	5.5
Butyric Acid *(%)*	.....	.....	0.13	0.11	0.61	1.24
Propionic Acid *(%)*	.....	.....	0.06	0.05	0.40	0.77

Within one set of averages the figures having the same letter as superscript form a statistically homogeneous group. Capital letters, $P = 0.01$; lower-case letters, $P = 0.05$.

were each fed to four Jersey heifers. Data on length of trial and D.M. content of the forages as harvested and as fed are given in the upper portion of Table 3. The heifers were placed in expected outcome groups based on age and previous performance and were randomly assigned to dietary treatment Groups 1, 2, or 3. Body weights were taken on three consecutive days at monthly intervals and at initiation and cessation of all trials. Trial II was terminated after 73 days, when the supply of haylage of feedable quality became exhausted.

In the paired-feeding trial (Trial IV) three groups of five Jersey heifers, each replicated as to age and previous rate of gain, were used. Heifers in Group A were fed alfalfa hay ad libitum, those in Group B were fed alfalfa silage ad libitum, and those in Group C were fed hay limited in amount to that of the

silage D.M. consumed by the respective silage-fed heifer in that replicate. This was done on a monthly basis so that Group C was fed at least one calendar month after Group B, although at the same chronological age. Three of the heifers in Group B were fed D.C. silage for 90 days, whereas the other two were fed wilted alfalfa silage for 125 days. Other information and data on initial age and weight are given in the upper portion of Table 4.

TABLE 4

Performance of heifers fed hay, silage, and limited hay

	Dietary treatment group***		
	A	B	C
Forage	Hay	Silage	Limit hay
No. of heifers	5	5	5
Days fed forage *(days—No. of heifers)*	90–3	90–3*	90–3
(days—No. of heifers)	125–2	125–2**	125–2
Initial age *(mo.) (age—No. heifers)*	18–3	18–3*	18–3
(age—No. heifers)	8–2	8–2**	8–2
Initial weight *(lb.)*	515–3	519–3*	477–3
	297–2	272–2**	295–2
Daily gain *(lb/calf)*	$0.93^{A} \pm 0.24$	$-0.10^{B} \pm 0.07$	$0.64^{A} \pm 0.24$
Daily intake *(lb. D.M/day)*	$11.59^{A} \pm 2.31$	$7.15^{B} \pm 2.38$	$6.83^{B} \pm 1.71$
Efficiency *(lb. gain/100 lb. D.M.)*	$8.23^{A} \pm 2.41$	-0.82 ± 4.17	$10.20^{A} \pm 5.41$

* D.C. silage.
** Wilted silage.
*** Groups B and C pair-fed—equal D.M. from silage or hay. Within one set of averages the figures having the same letter as superscript form a statistically homogeneous group. Capital letters, $P = 0.01$; lower-case letters, $P = 0.05$.

At all ages roughages were fed to the limit of appetite, except in Trial IV, Group A. Consumption and refusals were recorded daily. Dry matter (D.M.) content of the feeds fed in Trial I was determined two times per month by drying at 80° C. in a forced-draft oven. In Trials II, III, and IV the D.M. content of the silages was obtained at five- or ten-day intervals from daily aliquots by distillation from toluene. Other details were similar to those used in previous trials (2, 10, 13).

Standard statistical procedures were used as suggested by Snedecor and others (5, 9).

RESULTS AND DISCUSSION

Results on specific aspects of Trial I are presented and discussed, followed in order by Trials II, III, and IV. The cumulative body weights of the four groups in Trial I at monthly intervals are shown in Figure 1. Body weights at selected ages, gains, intakes, efficiency of gains, and statistically homogenous groupings for each breed and specific periods are given in Table 2.

Effect on body weight. At 5 mo. of age all groups of heifers in Trial I were approximately equal in respect to weight, intake, and rate of gain (Table 2; Figures 1 and 2). At this age, these heifers fed hay and silage were only slightly smaller than heifers fed hay in previous trials (1, 10, 12).

The subsequent body weights of heifers in Group 1 were equal to those observed previously, when only alfalfa hay was fed to heifers (1, 10, 13). The heifers in Group 4 (fed silage) grew at a much slower rate and were similar to those fed silage in previous experiments (4, 10, 12).

During the 5- to 12-mo. interval, the 20 heifers fed hay gained faster than the 27 heifers fed silage (1.27 vs. 0.68 lb/day; $P < 0.01$). During the 12- to 19-mo. age interval the 22 heifers fed hay also gained faster than the 25 heifers fed silage (1.40 vs. 0.79 lb/day; $P < 0.01$).

Reversing hay and silage feeding at 12 mo. of age to Groups 2 and 3 had a very marked effect on rate of body weight gain. During the 12-19 mo. age period heifers changed from silage to hay (Group 3) gained more than any other group ($P = 0.05$, breeds combined basis) and those changed from hay to silage (Group 2) gained the least.

Markedly increased rates of gain were observed for the heifers of Groups 1, 2, and 4 (except Group 1, Jerseys) when changed from experimental forage to hay, corn silage, and grain at 19 mo. of age. This increase did not occur in heifers of Group 3 which had previously been changed from silage to hay at 12 mo. of age.

The body weights at 2 yr. of age of Holstein heifers in Groups 2, 3, or 4 were 100 to 200 lb. less than that of heifers in Group 1. The body weights of Holstein heifers in Group 1 equaled those of the Beltsville standard (6) after 20 mo. of age, but the body weight of the Holstein heifers in Group 3 only equaled that of the standard by 2 yr. of age. Heifers of both breeds in Group 4 never equaled the standard.

The final weights of the Jersey heifers in Groups 1, 2, or 3 were approximately the same, but the weights of heifers in Group 4 were 130 lb. less. The equalization of body weights for Jersey heifers in Groups 1, 2, and 3 after 19 mo. of age was due to the elimination of the largest animal from Group 1 at 19 mo. and to a decrease in rate of gain of the remaining heifers in Group 1. A reduction in growth rate during this age interval has been noted previously in Jersey heifers reared on hay in this herd (10, 12).

After feeding an adequate ration for 5 mo. (19-24 mo. of age) complete recovery did not occur in heifers that had received wilted alfalfa silage for 14 mo. (5-19 mo. of age), nor did complete recovery occur in Holstein heifers that had received the silage during either of the 7-mo. periods. The conclusion appears justified that, under usual conditions, feeding wilted alfalfa silage as the only forage to dairy heifers for any 7- to 14-mo. interval during the growing period, and adequate rations at other times, will decrease their weight at 2 yr. of age. The weights at 2 yr. of age of heifers fed silage for 7-mo. periods, however, were not markedly reduced, thus indicating that silage may be usefully employed as the only feed for growing heifers for such periods, provided the heifers have an opportunity to recover from the effects of silage feeding sometime prior to calving.

Effect on intake. Differences in voluntary intake of heifers in Trial I paralleled those of gain (Table 1; Figures 1 and 2). During the 5- to 12-mo.

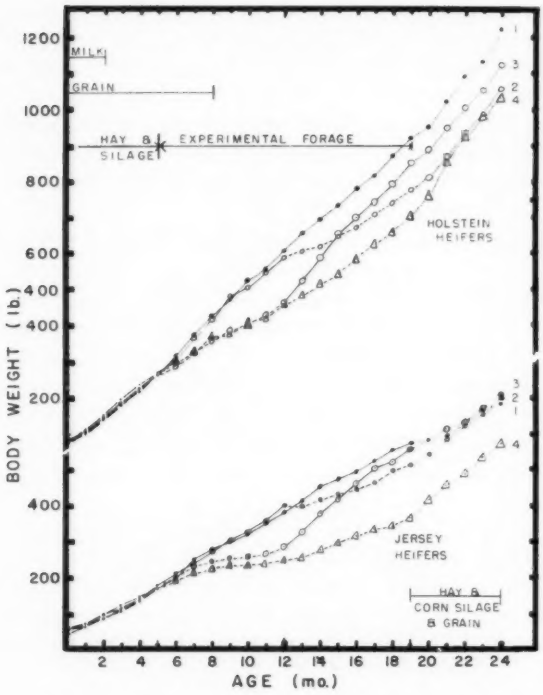


FIG. 1. Average body weights at monthly ages of heifers fed wilted alfalfa silage (dash line) or alfalfa hay (solid line), for given periods as outlined in text and in the figure.

age interval the heifers fed hay consumed more D.M. than those fed silage (9.81 vs. 6.84 lb/day; $P < 0.01$). During the 12- to 19-mo. age interval the heifers fed hay consumed more than those fed silage (15.47 vs. 10.68 lb/day; $P < 0.01$).

Heifers in Group 4 consumed the least of any group during the 12-19 mo. age interval in absolute terms, but in relation to body weight heifers in Group 2 consumed less than those in Group 4 (2.05 vs. 2.22 lb. D.M/cwt). Heifers in Group 3 consumed 2.85 lb. D.M/cwt, whereas those in Group 1 consumed 2.54 during this interval. During the recovery period, heifers in Group 4 consumed the most D.M. on a per-hundredweight basis (2.40 vs. 2.1 for other three groups), but on an absolute basis their consumption was lower than for most other groups.

Changing from hay plus silage to silage only at 5 mo. of age in Groups 3 and 4 produced a decrease or very small increase in total D.M. intake (Figure 2). This happened even though all the heifers were accustomed to eating relatively large quantities of silage during the pre-experimental period and rate of grain feeding did not change at this time. The authors believe that this reduction in intake is a reflection of the effect of silage on voluntary consumption not related to the palatability of the silage.

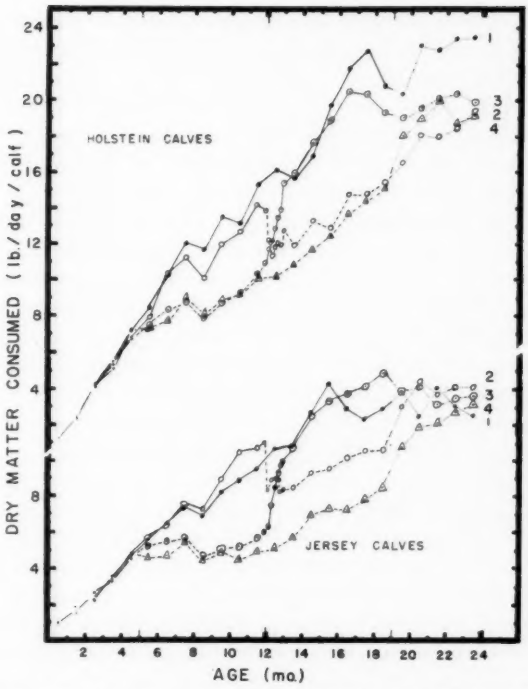


FIG. 2. Average daily dry matter consumed at monthly ages of heifers fed wilted alfalfa silage (dash line) or alfalfa hay (solid line), for given periods as outlined in text and in Figure 1.

An abrupt decrease in intake occurred when the heifers in Group 2 were changed from hay to silage, and a fast but not abrupt increase occurred for the heifers in Group 3 when they were changed from silage to hay at 12 mo. of age. These changes are plotted by five-day intervals for five days previous and for the month following this change in Figure 2. At both age intervals (5-12 and 12-19) the rate of increase in consumption as age increased was less for heifers fed silage than it was for heifers fed hay. The Jersey heifers in Groups 3 and 4 consumed no more D.M. when 10 to 12 mo. of age than when they were 6 mo. of age. Each group showed a temporary decrease in dry matter intake when grain was omitted at 8 mo. of age, and this temporary decrease was more evident in heifers fed silage than with most heifers fed hay.

The voluntary intake of silage as compared to hay was reduced in this trial, where the forage source was identical, just as it was in other trials where the source of the hay and silage was different (7, 10, 12).

Effect of efficiency of gain. The efficiency of body weight gains during the 5- to 12-mo. period was greater for the 20 hay-fed heifers than it was for the 27 silage-fed heifers (13.02 vs. 9.49 lb. gain/100 lb. D.M.; $P < 0.01$). However, during the 12- to 19-mo. period this was not always the case. The combined

breeds averaged 10.45 for Group 3; 8.56 for Group 4; 7.55 for Group 1; and 5.98 for Group 2 during this age interval. Reasons are not evident why Group 4, continued on silage, was significantly more efficient ($P < 0.01$ on a combined breed basis) than heifers in Group 2 which were changed to silage during this period. Heifers in Group 1 had a better appearance and were fatter than the heifers in Group 4; therefore, the caloric value of their gain was probably higher. This could account for the larger gain per unit of D.M. intake for heifers in Group 4 as compared to Group 1 during the 12-19 mo. age period. The efficiency of gain from 5 to 24 mo. was somewhat higher for heifers in Groups 3, 4, and 2 than for those in Group 1. These group differences were not significant. Breed differences in efficiency of gain were significant for the entire period, as well as for each experimental period ($P < 0.01$).

General effects. A significant effect of pretreatment was noted in the gains, intake, and efficiency for Group 3 during the 12-19 mo. age interval and for Groups 2 and 4 during the recovery period. These effects were characteristic of those obtained when changing from a low rate of intake to a more liberal rate of feeding.

Body weight gains, D.M. intake, and efficiency of gain were more variable for heifers receiving silage than for heifers receiving hay. This is indicated by the following coefficients of variation: (a) 34.6 and 10.7% for gains; (b) 13.8 and 12.7% for intake, and (c) 26.5 and 12.7% for efficiency of gain by silage- and hay-fed heifers, respectively. The greater variation among heifers fed silage than among heifers fed hay has been noticed previously (12). The greater variation in gains and efficiency with approximately equal variation in intake could indicate that there was a large difference in the way silage-fed heifers utilized the consumed forage.

The coefficients of variation for gain and efficiency were greater for Jersey heifers fed silage than for Holstein heifers fed silage (45 vs. 24% for gain and 36 vs. 17% for efficiency, respectively). There was no breed difference for heifers fed hay. In this and other trials there have been occasional Jersey heifers that when fed only wilted alfalfa silage weighed less than 400 lb. when 2 yr. of age. The reason for the very large variation in responses of heifers fed silage is not known.

Alfalfa from the same fields harvested as hay produced an average daily weight gain of 1.33 lb. in dairy heifers 5-19 mo. of age, whereas the same material harvested as wilted silage (averaging 31% D.M.) produced a daily gain of only 0.73 lb. The average daily intakes were 12.64 and 8.77 lb. D.M., respectively, whereas the average efficiencies were 11.01 and 8.44 lb. gain per 100 lb. D.M. consumed, respectively.

In Trial II the body weight gains, D.M. intake, and efficiency of gain were greatest for the heifers fed hay, least for those fed D.C. silage, and intermediate for those fed haylage (Table 3). In Trial II-b the heifers fed hay had larger gains and efficiency of gain than those fed D.C. silage. However, none of the dietary treatment differences was statistically significant, and replicates were significantly different only in D.M. intake and intake per hundredweight. This

was due to differences in ages of calves, which varied from 7 to 20 mo. in Trial II-a, and 16 to 22 mo. in Trial II-b. D.M. intake per unit body weight was less ($P < 0.05$) for heifers fed D.C. silage in Trial II-b, but there were no such differences due to treatment in Trial II-a.

In Trial III (1958 crop) the heifers fed hay and haylage were approximately equal in body weight gains, D.M. intake, and in efficiency of gain. However, four of the five heifers fed D.C. silage actually decreased in body weight during the trial. Those fed this D.C. silage had significantly lower rates of gain, intake, intake per hundredweight, and efficiency of gain ($P < 0.01$) than those fed hay or haylage (Table 3). In Trial III age of heifers varied from 15 to 19 mo. and there were no differences among replicate groups in any response studied.

In Trials II and III there were no appreciable differences in response of heifers fed heavily wilted silage (43-46% D.M.) and those fed the same material as hay. Those fed D.C. silage did not have as good a response and there was a very low response in heifers fed D.C. silage in Trial III compared to a reasonable response in heifers fed D.C. silage in Trial II. The reasons for the differences noted in the two D.C. silages are not known but presumably must be due to differences in the composition or other qualities of the two D.C. silages used. The D.M. of these two forages when ensiled were different. In Trial II it was 25.8% and in Trial III 20.5% D.M., and when fed the D.M. contents were 28 and 24%, respectively (Table 3). Apparently, the D.M. content of forage when ensiled has a large effect on both the subsequent fermentation process and acceptability of that silage by animals (2, 7, 12).

Some of the chemical constituents of the hay and silages are presented in the lower portion of Table 1. The two D.C. silages differed in their content of crude fiber, ammoniacal nitrogen, lactic, butyric, and propionic acids. Further studies are needed to determine if these and/or other constituents play a role in determining the extent of the response of heifers receiving silages.

In Trial IV the heifers fed limited amounts of hay had slightly lower D.M. intakes but significantly larger gains ($P < 0.01$) than did their pair-fed heifers receiving silage (bottom portion, Table 4). In this trial the lower rate of gain and higher efficiency of gain in heifers fed limited amounts of hay were not statistically significantly different from the values for heifers fed hay ad libitum (Table 4). Large individual variations were responsible for this lack of significance in these differences.

In this trial two of the heifers received wilted alfalfa silage and their pairmates received a commercial U.S. No. 1 alfalfa hay. The three other heifers received D.C. silage, whereas their pair mates received hay cut simultaneously from the same field. The same comparative result was obtained with each replicate group, so that these factors did not influence the significance of the differences observed. It is of interest to note that two of the three heifers fed D.C. silage decreased in body weight, whereas both heifers fed wilted silage had a slight increase (+ .08 lb/day) in body weight.

Although this trial may have certain inadequacies, the results definitely indicate that the nutritive value of dry matter from hay was superior to that

from either of the two silages used for growing dairy heifers at 8 or 18 mo. of age.

These three trials extend and confirm previous results indicating that heifers 7 to 20 mo. of age fed hay have a higher rate of growth and voluntary intake than contemporary heifers fed D.C. or regularly wilted silage, even when the forage was from the same source. Further, the trials demonstrate a difference in response to various silages. The silages made by wilting to more than 43% dry matter were practically equal to hay for growing heifers. The same material ensiled by the direct cut process was inferior to the high dry matter silage or hay. However, a large difference in comparative response between silages made in different years by the direct-cut procedure was demonstrated. It is suggested that the dry matter content of the forage at ensiling time and the resulting difference in fermentation are important factors in determining the acceptability of the resulting silage and the performance rate of animals fed the silage.

These and related factors affecting forage consumption by dairy animals are more fully discussed elsewhere (2, 7).

Heifers at any age studied (7 to 19 mo.) gained less when fed silage than when fed hay and the conclusion appears justified that age of heifer used did not alter the comparative performance when fed solely on hay or silage. The growth of heifers in Trial I up to 5 mo. of age indicate that silage can successfully be used even in the diet of very young calves when fed in addition to hay, grain, and milk. This is similar to the results of others (8). However, the suggestion mentioned earlier (10), that silage might be successfully used as the sole roughage for heifers over 1 yr. of age, was not confirmed in these investigations when D.C. or the usual wilted silages were fed.

REFERENCES

(1) Anonymous. High Roughage Rations for Holstein Calves, USDA-ARS, Production Research Rept. No. 15. 1957.

(2) Gordon, C. H., Derbyshire, J. C., Melin, C. G., Kane, E. A., Sykes, J. F., and Black, D. T. The Effect of Wilting on the Feeding Value of Silage. USDA, ARS 44-76. June, 1960. J. Dairy Sci., 43: 866. 1960.

(3) Headley, F. B. Value of Silage to Holstein Heifers. Nevada Agr. Expt. Sta., Ann. Rept. 1949.

(4) Keener, H. A., Allen, F. E., Colovos, N. F., Paul, A. C., and Davis, H. A. Value of Adding Corn Silage and Limited Quantities of Hay to a Grass Silage Limited-Grain Ration for Dairy Heifers. J. Dairy Sci., 41: 429. 1958.

(5) Le Clerg, E. L. Mean Separation by the Functional Analysis of Variance and Multiple Comparisons. USDA, ARS-20-3. 1957.

(6) Mathews, C. A., and Fohrman, M. H. Beltsville Growth Standard for Jersey Cattle and for Holstein Cattle. USDA Tech. Bull. 1098 and 1099. 1954.

(7) Moore, L. A., and Thomas, J. W. The Acceptability of Grass Legume Silage by Dairy Cattle. Proc. 8th Intern. Grassland Conf. 1960.

(8) Porter, G. H., and Kesler, E. M. Value of Alfalfa Silage in the Diet of the Young Dairy Calf. J. Dairy Sci., 40: 163. 1957.

(9) Snedecor, G. W. Statistical Methods. 5th ed. Iowa State College Press, Ames. 1957.

(10) Sykes, J. F., Converse, H. T., and Moore, L. A. Comparison of Alfalfa Hay and Alfalfa Silage as Roughage for Growing Dairy Heifers in a Limited Milk and Grain Feeding System. J. Dairy Sci., 38: 1246. 1955.

(11) THOMAS, J. W. Further Comparisons of Alfalfa Hay and Alfalfa Silage for Growing Dairy Heifers. USDA-ARS 44: 51. 1959. J. Dairy Sci., 42: 929. 1959.

(12) THOMAS, J. W., SYKES, J. F., AND MOORE, L. A. Comparison of Alfalfa Hay and Alfalfa Silage Alone and with Supplements of Grain, Hay, or Corn Silage for Growing Dairy Calves. J. Dairy Sci., 42: 651. 1959.

(13) WILSON, C., AND BARTLE, E. Alfalfa Silage versus Alfalfa Hay for Wintering Yearling Heifers. South Dakota Farm and Home Research, 5: (#1)4. 1953.

NUTRITIVE VALUE OF REED CANARYGRASS AS HAY WHEN GROWN WITH VARIOUS NITROGEN LEVELS[1, 2]

W. V. CHALUPA, J. L. CASON,[3] AND B. R. BAUMGARDT[4]

Department of Dairy Science, Rutgers—The State University, New Brunswick, New Jersey

SUMMARY

A total collection digestion trial was carried out with four dairy heifers in a 4 × 4 Latin-square extra-period design. The rations fed were second-cutting alfalfa hay and three reed canarygrass hays, the latter fertilized at the rates of 0, 100, or 200 lb. of nitrogen per acre. The reed canarygrass hay fertilized with 200 lb. of nitrogen per acre was equal or superior to the alfalfa hay in the digestibility of protein, fiber, ether extract, and energy, and in the amounts of TDN (%) and DE (cal/g). A close relationship between the DE and TDN methods of forage evaluation was expressed in the correlation coefficient of +0.736. Digestible energy can be expressed in terms of TDN through the linear regression equation $Y = 731.23 + 31.30X$ [Y = digestible energy (cal/g): X = total digestible nutrients (%)].

A roughage is considered the basic and most economical constituent of ruminant rations. While no single roughage is a complete and perfect ration, a high-quality palatable roughage containing adequate protein approaches this ideal. Much attention has been focused on improving yield, chemical composition, and digestibility of grass hays through nitrogen fertilization.

Many workers, as reviewed by Chalupa (5), have conducted experiments which indicate that applications from 100 up to 900 lb. of nitrogen per acre increase yield, percentages of crude protein and ether extract, and gross energy (cal/g); whereas, percentages of crude fiber, nitrogen-free extract (NFE), and ash are decreased slightly. Other workers (3, 12) have shown that the digestibility of protein, crude fiber, and ether extract, as well as total digestible nutrients (TDN) and digestible energy (DE), are increased with increasing applications of nitrogen.

Reed canarygrass (*Phalaris arundinacea*) is a highly adaptive grass (8) and there is little information available concerning its response to nitrogen fertilization. The following study was undertaken to determine the effect of nitrogen fertilization on the nutritive value of reed canarygrass hays, and the nitrogen balance of dairy heifers fed these hays. The TDN and DE methods of forage evaluation were compared for these hays.

Received for publication December 22, 1960.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station. Data presented are from a thesis submitted by the senior author in partial fulfillment of the requirements for a Master of Science degree.

[2] Supported in part by a grant from E. I. duPont de Nemours and Company.

[3] Present address: Department of Dairy Husbandry, University of Maryland, College Park.

[4] Present address: Department of Dairy Husbandry, University of Wisconsin, Madison.

EXPERIMENTAL PROCEDURE

Four 12-mo.-old purebred Holstein dairy heifers were used as experimental animals in an extra-period 4 × 4 Latin-square change-over design. The collections were carried out in digestion stalls constructed according to Horn *et al.* (9), with minor modifications.

The rations, fed twice daily at 8 A.M. and 5 P.M., were second-cutting alfalfa (serving as a comparison standard) and three reed canarygrass hays: (1) low-N = 0 lb. nitrogen per acre, first cutting; (2) medium-N = 100 lb. nitrogen per acre, second cutting; and (3) high-N = 200 lb. nitrogen per acre, second cutting. A ten-day preliminary period followed by a seven-day collection period was employed. During the first eight days of the preliminary period the animals were fed ad libitum to determine their maximum consumption. For the remainder of the experimental period they were fed 80% of their previous maximum intake.

Urine was collected in plastic carboys, containing H_2SO_4 and toluene, using a Size 20 Bardex hemostatic catheter which was inserted through the urethral orifice and inflated with 30 ml. of warm water (6). A sample (1% of the total volume) was composited daily and stored for analysis in a refrigerated jar containing H_2SO_4 and toluene. Feces fell directly into tared collection pans. Samples (1/40th of the total weight) were dried at 100 ± 1° C. for 24 hr. The daily fecal samples were then composited, allowed to air-equilibrate, ground, and stored in air-tight jars.

Proximate analyses were conducted as outlined by the Association of Official Agricultural Chemists (1, 2), with minor modifications. Gross energy was determined in a Parr Oxygen Bomb Calorimeter equipped with an adiabatic jacket (15).

The analyses of variance of the extra-period Latin-square design were conducted as outlined by Lucas (10), and the multiple range test according to Duncan (7). Correlation coefficients, regression coefficients, and the linear regression formula were calculated as outlined by Snedecor (18).

RESULTS AND DISCUSSION

The average chemical compositions of the roughages fed in the digestion trial are found in Table 1. Increased applications of nitrogen increased the crude protein (%), ether extract (%), and gross energy (cal/g), while crude fiber (%) and NFE (%) decreased. Other workers (3, 12, 17) reported similar results with grass hays.

TABLE 1

Average chemical composition of the rations (air-dry basis)

Rations	Crude Protein	Crude Fiber	Ether Extract	Ash	Moisture	N-free Extract	Gross Energy
	(%)						(cal/g)
Alfalfa	18.13	24.46	3.17	7.70	10.18	36.36	4,101
Reed Canarygrass 200 lb. N/A	21.64	22.27	4.68	6.22	9.55	35.64	4,255
Reed Canarygrass 100 lb. N/A	14.86	25.15	3.80	7.02	9.59	39.58	4,204
Reed Canarygrass 0 lb. N/A	12.72	26.80	3.25	6.68	9.95	40.60	4,071

The application of 200 lb. of nitrogen per acre increased the protein digestibility of reed canarygrass above that of the comparison standard, alfalfa (Table 2). The analysis of variance (Table 3) shows the direct effects due to treatment to be very highly significant ($P < 0.001$). The multiple range test (Table 4) indicates that the high-N fertilized reed canarygrass is more digestible than either low-N or medium-N reed canarygrass ($P < 0.01$) or alfalfa ($P < 0.05$). Barth (3) obtained similar results with reed canarygrass. Markley (12) and Poulton (16) reported increased protein digestibility with nitrogen-fertilized bromegrass and orchardgrass hays, respectively.

TABLE 2

Average apparent digestion coefficients, DE, and TDN of the rations (air-dry basis)

Rations	Crude Protein	Crude Fiber	Ether Extract	N-free Extract	Energy	TDN	DE
	(%)						*(cal/g)*
Alfalfa	73.22	42.65	43.36	67.94	57.19	54.02	2,346
Reed Canarygrass 200 lb. N/A	78.33	49.26	62.53	64.83	63.38	60.42	2,694
Reed Canarygrass 100 lb. N/A	67.45	61.82	48.80	60.61	57.26	53.63	2,407
Reed Canarygrass 0 lb. N/A	66.13	67.34	54.03	62.92	60.84	55.91	2,478

The differences in the digestibility of energy of the hays attributable to direct effects of treatments was significant at the 1-% level of probability (Table 3). The multiple range test (Table 4) indicates that the digestibility of the energy of the high-N reed canarygrass hay was greater ($P < 0.01$) than that of the alfalfa and medium-N reed canarygrass hays and greater ($P < 0.05$) than the low-N reed canarygrass hay.

In order to increase the nutritive value of a forage, it is desirable to increase the crude fiber digestion. However, nitrogen fertilization depressed the digestibility of fiber, even though a smaller percentage of this nutrient was observed

TABLE 3

Summary of the analyses of variance

Source of variation	(Mean squares) Period effects	Animal effects	Direct effects	Residual effects	Error
d.f.	4	3	3	3	5
Digestion coefficients (apparent)					
Crude protein	4.42	22.73[a]	144.37[c]	.47	3.42
Crude fiber	37.67	85.82[a]	514.29[b]	24.74	21.82
Ether extract	418.86	132.11	331.36	61.13	313.05
N-free extract	31.82	31.55	51.79	3.67	14.12
Energy	1.80	21.32[a]	35.71[b]	2.71	2.52
Digestible energy *(cal/g)*	2,985.98	32,619.92	97,460.77[a]	7,291.48	9,604.91
TDN *(%)*	18.01	11.76	36.24[a]	5.79	5.45

[a] Significant at the 5% level of probability.
[b] Significant at the 1% level of probability.
[c] Significant at the 0.1% level of probability.

with increasing applications of nitrogen. Apparently, nitrogen fertilization depressed the more-digestible fraction of the fiber, but there was no change in the less-digestible fraction. Barth *et al.* (3) obtained similar results while Markley *et al.* (12) found a slight increase in crude fiber digestibility attributed to nitrogen fertilization.

TABLE 4

Multiple range test

Source of variation	Significant, 1% level	Significant, 5% level
Digestion coefficients (apparent)		
Crude protein	H > L, M; A > L	H > A; A > M
Crude fiber	L, M > A, H	
Energy	H > A, M	H > L; L > M, A
Digestible energy *(cal/g)*	H > A, M	H > L
TDN *(%)*	H > L > M, A	

The digestibility of ether extract and NFE showed no consistent trends in this study. This may be due to errors inherent in the methods: ether extract of the feces may have contained metabolic fat as distinguished from undigested or absorbed fat; NFE is computed as a difference and may, therefore, contain accumulated errors.

The TDN (%) and DE (cal/g) values for the hays are shown in Table 2. The analysis of variance (Table 3) indicated that the direct effects due to treatment upon DE and TDN are significant ($P < 0.05$). From the multiple range test, it is apparent that the high-N fertilized reed canarygrass is superior to the medium-N and low-N reed canarygrass hays and to the comparison standard alfalfa ($P < 0.01$) in terms of DE and TDN.

Digestible energy is considered to be the limiting factor in the nutritive value of forages. Swift (19), Barth *et al.* (4), Macdonald (11), and Markley *et al.* (12) indicate there is a close relationship between DE and TDN. Both methods of forage evaluation essentially measure feedstuffs to the same degree of availability to the animal body. Although TDN is the more common approach, it is time-consuming, indirect, and empirical. In this study, the relationship of the individual TDN and DE values is expressed by the correlation coefficient of +0.736, which is significant at the 1-% level of probability. DE can be expressed in terms of TDN through the linear regression formula:

$$Y = 31.30X + 731.23 \; [Y = \text{DE; (cal/g) and } X = \text{TDN (\%)}].$$

The four animals gained weight during the trial. This gain in weight would possibly indicate that the animals were in nitrogen equilibrium. Maynard and Loosli (13) state that it is important that the animals used for measuring the maintenance requirement by the balance technique be in a good state of nutrition at the start, because the requirement arrived at should be sufficient to maintain the protein reserves, not merely to balance losses from the body. The

heifers were in positive nitrogen balance throughout the experiment, but the results of the individual periods of the Latin-square design were quite variable and appeared to be confounded in some way. Since all four heifers were on identical roughage and grain rations prior to the initiation of this study, the following data obtained during the first period of the Latin-square design might be a better indication of the nitrogen retention than averages of all experimental periods: alfalfa = +37.97 g/day, reed canarygrass 200 lb. N/A = +14.85 g/day, reed canarygrass 100 lb. N/A = +9.20 g/day, reed canarygrass 0 lb. N/A = +4.35 g/day.

REFERENCES

(1) Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. 6th ed. Washington, D. C. 1945.

(2) Association of Official Agricultural Chemists. Official Methods of Analysis. 8th ed. Washington, D. C. 1955.

(3) Barth, K. M., Vander Noot, G. W., and Cason, J. L. A Comparison of the Nutritive Value of Alfalfa Hay with Bromegrass and Reed Canarygrass Hays at Various Levels of Nitrogen Fertilization. J. Nutrition, 68: 383. 1959.

(4) Barth, K. M., Vander Noot, G. W., and Cason, J. L. The Quantitative Relationship Between Total Digestible Nutrients and Digestible Energy Values of Forages. J. Animal Sci., 18: 690. 1959.

(5) Chalupa, William V. The Nutritive Value of Reed Canarygrass Hays Grown Under Different Levels of Nitrogen Fertilization. Master of Science thesis, Rutgers, The State University. 1959.

(6) Cunningham, H. H., Frederick, G. L., and Brisson, G. T. Application of an Inflatable Urethral Catheter for Urine Collection from Cows. J. Dairy Sci., 38: 997. 1955.

(7) Duncan, David B. Multiple Range and Multiple F Tests. The New Multiple Range Test. Biometrics, 11: 2. 1955.

(8) Heath, M. E., and Hughes, H. D. Reed Canarygrass. Forages. The Iowa State College Press, Ames. p. 317. 1951.

(9) Horn, L. H., Ray, M. L., and Neuman, A. L. Digestion and Nutrient-Balance Stalls for Steers. J. Animal Sci., 40: 225. 1957.

(10) Lucas, H. L. Extra-Period Latin-Square Design. J. Dairy Sci., 40: 225. 1957.

(11) Macdonald, G. J. A Comparison of the Total Digestible Nutrients and the Digestible Energy Method of Feed Evaluation Utilizing Alfalfa Hay and High Nitrogen Orchardgrass Hays. Master of Science thesis, Rutgers, The State University. 1959.

(12) Markley, R. A., Cason, J. L., and Baumgardt, B. R. Effect of Nitrogen Fertilization or Urea Supplementation upon the Digestibility of Grass Hays. J. Dairy Sci., 42: 144. 1959.

(13) Maynard, L. A., and Loosli, J. K. Animal Nutrition. 4th ed. McGraw-Hill Book Company, Inc., New York. 1956.

(14) Morris, M. P., and Gonzalez-mas, Alma. Simple Colorimetric Method for the Determination of Nitrates in Forage Crops. J. Agr. Food Chem., 6: 456. 1958.

(15) Parr Manual No. 120. Oxygen Bomb Calorimetry and Oxygen Bomb Combustion Methods. Parr Instrument Co., Moline, Illinois. 1948.

(16) Poulton, B. R., Macdonald, G. J., and Vandernoot, G. W. Effect of Nitrogen Fertilization on the Nutritive Value of Orchardgrass Hay. J. Animal Sci., 14: 462. 1957.

(17) Ramage, C. H., Eby, C., Mather, R. E., and Purvis, E. R. Yield and Chemical Composition of Grasses Fertilized Heavily with Nitrogen. Agronomy J., 50: 59. 1958.

(18) Snedecor, G. W. Statistical Methods Applied to Experiments in Agriculture and Biology. 5th ed. The Iowa State College Press, Ames. 1956.

(19) Swift, R. W. The Nutritive Evaluation of Forages. Northeast Regional Publ., No. 27, Pennsylvania State University, Bull., 615: 1. 1957.

LIVABILITY OF PUREBRED VS. CROSSBRED DAIRY CATTLE[1]

F. N. DICKINSON
Dairy Husbandman, Agricultural Research Service, USDA

AND

R. W. TOUCHBERRY
Department of Dairy Science, University of Illinois
Urbana

SUMMARY

The data for this study were obtained from the Illinois Dairy Cattle Crossbreeding Project. Only those causes of death were included in the analysis which were considered to reflect on the viability, or to show a possible sign of weakness of a breed group. Deaths due to ingesting hardware and poisoning were excluded. Significant differences in livability between the purebreds and the crossbreds or ¾-breds were found in first-generation heifers from birth to 1 yr. of age and cows over all lactations, and in second-generation heifers from birth to 1 yr. of age and from birth to calving age. In each of these cases, over twice as many purebreds were lost from the herds as crossbreds or criss-crosses. The over-all death losses in both generations were 32.7% for purebreds and 13.4% for the crossbreds and ¾-breds. The mean milk production of the live vs. dead cows in each breed group of the first generation was analyzed by the method of least squares. A small and nonsignificant difference was found between the production of the live and dead animals when considered over all animals regardless of breed, by breed of sire, by breed of dam, or by system of mating (purebreeding vs. crossbreeding). This indicates that there is little genetic relationship between livability and productive level and emphasizes the importance of improving the livability characteristics of dairy animals, so that more discretion can be exercised by the dairyman in culling cows. Most studies have indicated only a small or no influence of additive genetic variation on livability characteristics and, therefore, little chance of improving these characteristics by mass selection. However, this study indicates a considerable influence of nonadditive genetic factors, or heterosis, on livability and that crossbreeding dairy cattle may provide a means for immediate and marked improvement.

Studies on death losses (6, 7, 11, 14, 18, 21, 23) have shown that from 6 to 18% of all conceptions result in full-term stillbirths or nonviable abortions. Other studies (1, 3, 13, 14, 15, 17, 18, 21, 22, 23) have reported that of all calves alive at birth from 8 to 24% die before 1 yr. of age. It is reported (1, 14) that during the period from 1 yr. of age to calving from 7 to 9% of all heifers die or leave the herd because of failure to breed. From these reports, it can be shown that of all conceptions approximately 12% result in abortions or stillbirths and that of all heifer calves born, from 7 to 35% fail to breed or die before producing a calf. In these studies, the average death loss of calves born was approximately 20%.

Received for publication January 9, 1961.

[1] The data for this study were taken from the Dairy Cattle Crossbreeding Project being conducted at the Illinois Agricultural Experiment Station. This project is a cooperative project between the Dairy Cattle Research Branch of the ARS, USDA, and the Department of Dairy Science of the University of Illinois, and is a contributing project to Regional Project NC-2, The Improvement of Dairy Cattle Through Breeding.

Studies on dairy cows (2, 4, 5, 12, 19) have shown that the major causes of loss have been udder and reproductive difficulties. It has not been uncommon for these two causes to result in the eventual removal of from one-third to one-half of the cows from a herd. In general, no one breed of cows appears to have a marked advantage in terms of livability.

Workers who have investigated the heritability of longevity or fertility (8, 9, 16, 19, 20) have concluded that there is little chance of improving these characteristics through mass selection among cows that have calved.

The purpose of the present study is to determine if livability in dairy cattle is influenced by crossbreeding. The term livability as used in this study indicates the ability of a cow to stay in a herd without being removed by death, disease, or other debilitating abnormalities. Thus, in this study, animals that were removed from the herd because of sterility, udder troubles, etc., were classified as having had poor livability, along with those animals that actually died.

SOURCE AND DESCRIPTION OF DATA

The data in this study were taken from first- and second-generation female progeny in the Dairy Cattle Crossbreeding Project at the Illinois Agricultural Experiment Station. The first generation consisted of purebred Holsteins and Guernseys and two groups of reciprocal crossbreds. Animals in all four breed groups were progeny of the same foundation group of purebred Holstein and Guernsey dams. Each of the sires used had contemporary purebred and crossbred offspring in the herd. The second generation consisted of purebred Guernseys and Holsteins, 3/4 Holstein–1/4 Guernsey, and 3/4 Guernsey–1/4 Holstein progeny and were all progeny of first-generation cows and unrelated purebred Holstein and Guernsey bulls. Both generations have been included in the calf study, but only first-generation cows in the cow study, because so few second-generation cows have completed more than one lactation.

The experiment was designed in such a way that the main difference between the purebreds as a group and the crossbreds or criss-crosses as a group was the manner in which the germ plasm of the animals in each group was derived, either through purebreeding or crossbreeding. The mating plan and conduct of the experiment has tended to randomize the effects of additive genetic variation and environment over purebreds and crossbreds, leaving any differences between the purebreds and crossbreds or criss-crosses due mainly to the effects of nonadditive genetic influences, i.e., heterosis.

ANALYSIS OF DATA AND RESULTS

Calf losses. The calf data consist of two distinct sets, one from each of the first two generations. Death losses in these data have been separated into two categories, those reflecting in any way a sign of weakness or lack of viability of the breed group, and those that are strictly accidental deaths, apparently due entirely to chance. In these first two generation groups, three deaths were put in the latter category, one calf that was poisoned, one that caught her neck in a feed bunk and strangled, and one calf that died of hardware penetration of

the heart (traumatic pericarditis). The number of calves that died in each breed group from causes that may reflect a weakness of that breed group are listed and categorized according to cause of death in Table 1. Each death attributed to a specific disease or abnormality is supported by veterinary diagnosis. In the single case where the cause of death was not determined, post-mortem examination ruled out poisoning and hardware. It is interesting to note the shortage of purebred Guernseys in the second generation. This is due in part to the higher death losses among the first-generation Guernseys.

TABLE 1

Causes and per cent of calf losses by breed groups in the first two generations

Cause of removal from herd	First generation				Second generation			
	H	H♂ G♀	G♂ H♀	G	H	¾ H	¾ G	G
Total born	35	30	33	30	58	53	48	24
Total died	5	2	5	10	11	3	4	5
Per cent died	14.3	6.7	15.2	33.3	19.0	5.7	8.3	20.8
Born dead		1		4	4	1		1
Weak at birth	1				1		2	1
Pneumonia			1	3	2		1	2
Gastroenteritis	1			1				
Peritonitis		1		1				
Sterile	2		4		2	1		
Freemartin				1	1		1	1
Parasites	1							
Hypoplasia of cerebellum						1		
Cause undetermined					1			

The results of the Chi-square test of differences in death losses between first-generation purebreds and crossbreds and between second-generation purebreds and ¾-breds are shown in Table 2. In each generation, over twice as many purebreds as crossbreds have been lost prior to calving, even though in the second generation there were about 20% more ¾-breds born than purebreds. These differences in the second generation are significant at the 2% level. This rather large difference in death rate seems to reflect a definite inability of the purebreds to survive to calving age, as well as the crossbred heifers, even under the fairly high level of environment in which they were raised. It is possible that in a less desirable environment than was provided for these animals, the difference in losses between the purebreds and the crossbreds and criss-crosses might have been greater than was found in this study. It appears that the crossbreds are particularly more able to withstand the rigors of birth and the common calfhood diseases. The difference in losses up to 1 yr. of age is significant at the 5% level in both generations, favoring the crossbred heifers in each case. It can be noted in Table 2 that except for the 24-mo. and older age bracket in the first generation, the number of purebreds lost in each age group is larger than the number of crossbreds, in both generations. To date, in the third generation, ten out of 55 purebreds and four out of 55 ⅝-breds have been lost. This difference is significant at the 10% level of probability. Thus, the trend in these data is continuing so far in the third generation.

TABLE 2

Losses from death, sterility, etc., of purebreds, crossbreds, and ¾-breds

	Total at start of period	Number dead	Per cent dead	χ^2	Significance level
First generation					
Birth to calving					
Purebreds	65	15	23.1	3.22	.10
Crossbreds	63	7	11.1		
Birth to 12 mo.					
Purebreds	65	10	15.4	3.96	.05
Crossbreds	63	3	4.8		
12 to 24 mo.					
Purebreds	55	3	5.5	1.24	.30
Crossbreds	60	1	1.7		
24 mo. and older					
Purebreds	52	2	3.8	0.10	.75
Crossbreds	59	3	5.1		
Second generation					
Birth to calving					
Purebreds	82	16	19.5	6.51	.02
¾-breds	101	7	6.9		
Birth to 12 mo.					
Purebreds	82	11	13.4	4.06	.05
¾-breds	101	5	5.0		
12 to 24 mo.					
Purebreds	71	2	2.8	0.11	.75
¾-breds	96	1	1.0		
24 mo. and older					
Purebreds	69	3	4.3	1.83	.20
¾-breds	95	1	1.1		

Cow losses. The death losses among first-generation progeny that produced at least one calf before leaving the herd are shown in Table 3. Thirty-one per cent of the Holstein cows and 42.1% of the Guernsey cows were removed from the herd because of some sickness or disease, whereas only 16.0 and 14.8% of the two crossbred groups, respectively, have been lost from similar causes. Four cases of death from hardware have been omitted. Tests of the differences in death losses between purebred and crossbred first-generation cows are shown in Table 4. The difference in death losses over all lactations is significant at the 2.5% level in favor of the crossbreds. This is an indication that the trend found in the calf data apparently continues all the way through life, with the crossbreds being decidedly less susceptible to sickness, disease, and reproductive difficulties than are the purebreds.

Upon finding a significant difference in the rate of death loss between the purebred and crossbred cows, the question of the relationship between level of production and susceptibility to death presented itself. The importance of the difference in livability of the crossbred and purebred cows is really dependent on the relative level of production of the cows that tend to be lost from each group. To estimate this relationship, the milk production of all cows included in Table 3 that had completed at least one record was analyzed by the method of least squares. The cows were first subclassified within breed groups according

TABLE 3

Losses among first-generation progeny that calved at least once before leaving herd

	Holsteins	H ♂ × G ♀	G ♂ × H ♀	Guernseys
Total cows	29	25	27	19
Total leaving herd for cause	9	4	4	8
Per cent leaving herd for cause	31.0	16.0	14.8	42.1
Reasons for leaving herd				
Reproductive difficulties	3	1	1	4
Mastitis	2	2		1
Bloat	2	1		
Tuberculosis	1			
Lameness	1		1	
Brucellosis			1	
Pneumonia				2
Undetermined			1*	1**

* Died suddenly—no hardware or symptoms of poisoning found.
** Became sick after calving, lost weight, milked poorly.

to whether they had left the herd for the causes listed in Table 3. To facilitate terminology, the cows leaving the herd for causes listed in Table 3 are called the dead group, and the remaining cows, the live group.

The relative productive level of each cow was estimated by weighting the mean of her 2 ×, 305-day M.E. milk records by the inverse of the variance of the mean of those records. The weighted subclass means were then used to calculate the least-squares constants, using the orthogonal comparisons derived from the model

$$y_{ijkl} = \mu + a_i + b_j + ab_{ij} + c_k + ac_{ik} + bc_{jk} + abc_{ijk} + e_{ijkl}$$

where y_{ijkl} is the weighted mean of the records of any cow, a_i is the breed of sire (Holstein or Guernsey), b_j is the breed of dam (Holstein or Guernsey), and c_k represents the two subgroups within each breed group, live group, and dead group. The standard error of these constants was calculated according to the method described by Harvey (10). In brief, this consists of calculating the appropriate diagonal element of the inverse of the matrix of subclass numbers. The standard error of any constant is then the square root of the product of the appropriate diagonal element of the inverse matrix times the appropriate pooled error variance. The pertinent results of the least-squares analysis are shown in Table 5.

Table 5 reveals almost no difference in milk production between the live and dead groups, among either the Holsteins or Guernseys. The differences between the live and dead groups in the crossbreds is apparently due to sampling errors, which would probably be quite large in these two groups, due to the small number of cows that died. The constants in Table 5 indicate that no difference in level of milk production between the live and dead groups was detected when considered over all animals regardless of breed group, by breed of sire, by breed of dam, or by system of mating. In other words, this analysis points out the random manner in which premature death has struck the high- and low-producing cows of this project.

TABLE 4

Death losses among first-generation purebred vs. crossbred cows

	Purebreds	Crossbreds	χ^2	Significance level
Number calved at least once	48	52		
Number leaving herd for cause in all lactations	17	8	5.34	.025
Per cent	35.4	15.4		
Losses in Lactations 1 and 2				
Number starting first lactation	48	52		
Number leaving herd for cause	5	2	1.66	.20
Per cent	10.4	3.8		
Losses in Lactations 3 and 4				
Number starting third lactation	43	50		
Number leaving herd for cause	9	4	3.22	.10
Per cent	20.9	8.0		
Losses in Lactations 5 and 6				
Number starting fifth lactation	34	46		
Number leaving herd for cause	3	2	.67	.50
Per cent	8.8	4.3		

TABLE 5

Weighted subclass means and constants for least-squares analysis of relation between level of milk production and death losses

Breed group	Weighted mean milk production *(lb.)*
Holsteins	
Live	12,931
Dead	12,954
Crossbreds (H ♂ × G ♀)	
Live	9,701
Dead	10,505
Crossbreds (G ♂ × H ♀)	
Live	8,128
Dead	7,422
Guernseys	
Live	5,193
Dead	5,315

Effect*	Constant	S.E.
c_k	−30	246
ac_{ik}	−176	246
bc_{jk}	201	246
abc_{ijk}	−6	246

* a_i = Breed of sire (Holstein or Guernsey).
b_j = Breed of dam (Holstein or Guernsey).
c_k = Live group vs. dead group.

DISCUSSION OF RESULTS

The importance of dairy cows being able to live a long, healthy, and profitable life is unquestionable, whether it be on the ubiquitous family farm or in the specialized milk-factory system found on the West Coast. This does not necessarily mean that all cows should be kept in the herd for as long as possible, since rate of genetic progress through selection is in part a function of generation length and selection differential. The real place for gain through greater livability is in reducing the rate of loss of animals from inherent weakness and susceptibility to disease and thereby increasing the proportion of cows that can be advantageously culled as part of a selection program. The review of the literature on death losses has indicated that heritability of longevity and fertility among cows that calved is practically zero. This would indicate little additive genetic influence over these characteristics and probably little hope for improvement through mass selection for longevity. However, the results of this study indicate that the influence of nonadditive genetic factors may well provide a means for considerable and for quite rapid improvement in the livability characteristics of dairy cattle. An examination of Table 1 indicates that well over half of the losses incurred before calving age resulted from failure to survive birth or inability to withstand the common calfhood diseases. This is probably a representative picture of conditions throughout the industry. The early part of life for most animals is the most crucial from the point of view of probability of survival. As seen in Table 2, crossbreeding has resulted in a significantly lower death rate than purebreeding up to 12 mo. of age in both generations. The genetic diversity resulting from crossbreeding seems to have imparted a significantly higher survival rate on the crossbred calves. Between the age of 1 yr. and calving age, the principal cause of death loss in this project has been reproductive difficulties. This again is probably typical of the industry as a whole. There is little difference between purebred and crossbred heifers in losses due to reproductive troubles. However, reproductive difficulties constituted the largest single cause of the significant difference in death losses between purebred and crossbred cows.

A comparison of the number of calves born in the purebred and crossbred groups, and the number of cows surviving through the sixth lactation, will demonstrate vividly what the advantage in livability enjoyed by the crossbreds would mean to a dairyman. Of 65 purebred heifer calves born, only 31 survived through the sixth lactation. Of 63 crossbred heifers born, 44 survived through the sixth lactation. Perhaps a more practical consideration would be to consider just the first and second lactations, since the average cow in the United States completes between two and three lactations. Of 65 purebred heifers born, 20 failed to complete two lactations for causes which reflect a sign of weakness on the breed group, while only nine of 63 crossbreds were lost for the same reasons. This difference is significant at the 5% level. From the purely practical aspect, this means that the dairyman with a crossbred herd would have lost only about one-half as many animals due to weakness and dis-

ease as the dairyman with purebreds and, therefore, would have been able to cull many more animals as part of a selection program.

When the figures in Table 5 are considered, the differences in death losses of cows shown in Table 3 assume added significance. The figures in Table 5 indicate that death from weakness and disease seems to strike the higher producers and lower producers in a more or less random manner. Thus, death is about as likely to remove one of the cows that would normally be kept in the herd as it is to remove one of the cows that would be culled. Therefore, it is of primary importance to decrease death losses and give the dairyman more of a chance to cull the cows that should be culled and still maintain his herd size.

A flat recommendation should not be made to purebred breeders that they crossbreed in order to increase the livability of their stock. Obviously, the economics of losing the purebred identity of a herd would in many cases more than offset the advantage of the more hardy crossbred animals. However, from the consideration of herd health and longevity, there would seem to be a real place for crossbreeding in the management program of the average commercial dairyman keeping a grade herd. This implies a man whose cows have no value over beef prices on the open market. This category would include the majority of dairymen in this country.

REFERENCES

(1) Arnold, P. T. D., and Becker, R. B. Dairy Calves; Their Development and Survival. Florida Agr. Expt. Sta., Bull. 529. 1953.

(2) Asdell, S. A. Variations in Amount of Culling from DHIA Herds. J. Dairy Sci., 34: 529. 1951.

(3) Atkeson, F. W. Calf Losses in the University Herd. Idaho Agr. Expt. Sta., Bull. 179. 1931.

(4) Baltzer, A. C. Causes for Cow Removals in Michigan Herds Under Test in Dairy Herd Improvement Associations. Michigan Agr. Expt. Sta., Quart. Bull. 22. 1940.

(5) Becker, R. B., Arnold, P. T. D., and Spurlock, A. H. Productive Life-Span of Dairy Cattle. Florida Agr. Expt. Sta., Bull. 540. 1954.

(6) Brody, A. G., Hogan, A. G., Kempster, H. L., Ragsdale, C. C., and Trowbridge, E. A. Growth and Development with Special Reference to Domestic Animals. I. Quantitative Data. Missouri Agr. Expt. Sta., Research Bull. 96. 1926.

(7) Davis, H. P. Dairy Calf Births and Disposals, University of Nebraska Herd, 1904-1948. Nebraska Agr. Expt. Sta., Bull. 411. 1952.

(8) Dunbar, R. S., Jr., and Henderson, C. R. Heritability of Fertility in Dairy Cattle. J. Dairy Sci., 33: 377. 1950.

(9) Dunbar, R. S., Jr., and Henderson, C. R. Heritability of Fertility in Dairy Cattle. J. Dairy Sci., 36: 1063. 1953.

(10) Harvey, W. R. Least-Squares Analysis of Data with Unequal Subclass Numbers. ARS-20-8. USDA. 1960.

(11) Herman, H. A., Spalding, R. W., Hineman, J. H., and Bower, K. W. Reproductive Efficiency in Dairy Cattle. Missouri Agr. Expt. Sta., Research Bull. 528. 1953.

(12) Horwood, R. E. Loss of Cows from Dairy Herds Reduces Income. Michigan Agr. Expt. Sta., Quart. Bull. 31. 1949.

(13) Horwood, R. E., and Weaver, E. What Becomes of the Calves in Purebred Dairy Herds? Michigan Agr. Expt. Sta., Quart. Bull. 32. 1949.

(14) Ingels, J., and Cannon, C. Y. The Mortality of Calves in the Iowa State College Dairy Herd. Proc. Am. Soc. Animal Production. p. 223. 1936.

(15) JOHNSON, R. E., JUNGHERR, E. F., AND PLASTRIDGE, W. N. Calf Losses in a Self-Contained Herd Over a Period of Seventeen Years. J. Dairy Sci., 31: 669. 1948.

(16) LEGATES, J. E. Genetic Variation in Services per Conception and Calving Interval in Dairy Cattle. J. Animal Sci., 13: 81. 1954.

(17) MILLER, K., AND GILMORE, L. Calf Mortality, Sex Ratio, and Incidence of Twinning in Two University of Minnesota Herds. J. Dairy Sci., 32: 706. 1949.

(18) ORMISTON, E. E. Calf Losses in a Dairy Herd Consisting of Five Breeds. J. Dairy Sci., 32: 712. 1949.

(19) PARKER, J. B., BAYLEY, N. D., FOHRMAN, M. H., AND PLOWMAN, R. D. Factors Influencing Dairy Cattle Longevity. J. Dairy Sci., 43: 401. 1960.

(20) POU, J. W., HENDERSON, C. R., ASDELL, S. A., SYKES, J. F., AND JONES, R. C. A Study of the Inheritance of Breeding Efficiency in the Beltsville Dairy Herd. J. Dairy Sci., 36: 909. 1953.

(21) SAVAGE, E. S., AND MCCAY, C. M. The Nutrition of Calves; A Review. J. Dairy Sci., 25: 595. 1942.

(22) WEAVER, E., HORWOOD, R. E., AND SMILEY, E. S. Losses of Calves in Dairy Herds. Michigan Agr. Expt. Sta., Quart. Bull. 32. 1949.

(23) WING, H. H. The Cornell University Dairy Herd, 1889 to 1928. Cornell University Agr. Expt. Sta., Bull. 576. 1933.

PROGESTINS IN CORPORA LUTEA, OVARIES, AND ADRENALS AFTER ESTRUS AND BREEDING OF NORMAL AND ABNORMAL COWS [1]

R. E. ERB AND F. STORMSHAK [2]

Department of Dairy Science, Washington State University, Pullman

SUMMARY

Progesterone and Δ^4-pregnene-20 β-ol-3-one (progestins) were separately determined in the corpus luteum, ovaries, and adrenals. A total of 77 animals was studied and 53 were considered reproductively normal at the time of slaughter. Progestin content of the corpus albicans prior to ovulation ranged from 0 to 24 μg. Progestin concentration per gram was high in the developing corpus luteum on the second and third days of the cycle, but the total quantity was generally less than 100 μg. until after the sixth day. Maximum quantities were observed at 14-16 days (six cows averaged 251 μg.). Decline in concentration and total progestins was not marked until near the time of an expected estrus. During pregnancy, the average level for seven cows was 161 μg. at 25-34 days and for six cows, 250 μg. at 37-42 days. The differences were also reflected in concentration, since the weight of the corpus luteum was, on the average, essentially constant. Neither size nor progestin content of the ovaries and adrenals varied appreciably among these cows. Adrenals of four cows 8-22 days post-partum were large (average 46 g.) and contained about four times more progestins than is generally observed (66 μg.). The progestin content of the corpus luteum of repeat breeders was not unlike normal animals in a similar stage of the estrous cycle. The corpora lutea and fluids from cystic follicles generally had less progestins than luteal cysts or the fluid they contained. One cow with a dead embryo at 20 days postbreeding had only 8 μg. of progestins in the corpus luteum.

Edgar and Ronaldson (2) observed that progesterone concentration in ovarian venous blood of ewes increased most rapidly from Days 4-7 and peaked on Day 14 of the estrous cycle. The quantity of progesterone in the blood leaving the ovary of the ewe, at least during the proliferative stages of the corpus luteum (CL), coincides well with its increase in size and histological evidence of secretory activity. Though the histological changes in the CL have been described in some detail for the cycling (1) and early pregnant (3) cow, relatively little is known about how these changes coincide with progesterone levels. Progesterone concentration is over 30 μg/g of CL at the 14th day in cycling (5, 16) and pregnant cows (16), and these levels decline by 28 days (16). Progesterone and Δ^4-pregnene-20 β-ol-3-one (20 β-ol) are significantly higher in CL from 16 to 89 (33.6 μg/g) than at 90 to 179 (20.4 μg/g) or after 180 days (27.5 μg/g) of pregnancy (14). The present work was undertaken to quantitatively estimate progestin levels in CL ovaries and adrenals of cows at intervals after estrus and breeding. During the study, cows with abnormal reproduction were also available for study and these are reported for comparative purposes.

Received for publication December 17, 1960.

[1] Scientific Paper No. 2055, Washington Agricultural Experiment Stations, Project 1359. This investigation was supported in part by funds for biological and medical research by the State of Washington, Initiative Measure No. 171.

[2] Present address: Department of Genetics, University of Wisconsin, Madison.

MATERIALS AND METHODS

Ovaries and adrenals of cows with known breeding histories were removed at the time of slaughter. The CL was removed from the ovary bearing it and the tissues were separately frozen in solid CO_2 and stored at $-25°$ C. until prepared for assay. A total of 77 animals was studied and 53 were considered reproductively normal at the time of slaughter. Thirteen of the animals were from Kansas State University and eight were from the University of Idaho dairy herds. Ovaries and adrenals were not available for the Kansas cows. Adrenals were not available for three Idaho cows.

In cases where cows were bred at the last estrus, the reproductive tracts were flushed with distilled water and the contents examined under a wide-field dissecting microscope.

Progesterone and 20 β-ol (progestins) were separately determined in CL and the combined ovaries and adrenals of individual cows, using the method of Stormshak *et al.* (15).

RESULTS AND DISCUSSION

Estrous cycle. Among four cows slaughtered after estrus but before ovulation, three had corpus albicans from the previous cycle and two of these contained measurable quantities of progestins (Table 1). All had follicles near 18 mm. in diameter. By two to three days after start of estrus the developing CL varied from 0.7 to 1.7 g. and the average concentration of progestins was 69 μg/g. At four to five days (three cows) the CL had doubled in weight, but the concentration was only 28.5 μg/g and the total progestins were lower than at two to three days. Though not shown in Table 1, the concentration and total progestins were distinctly lower in two of the three cows at three and four days than for two cows at two days and two cows at five days. These differences may be caused by delayed ovulation or by changes in development. According to Cupps *et al.* (1), the cells of the theca interna reach maximum size at estrus and appear to regress without histological signs of luteinization until eight days after estrus. The granulosa cells enlarge during heat and become very compact and show signs of luteinization on the second day of the cycle. By the sixth day some are fully luteinized. By Day 12 luteinization of theca and granulosa cells was complete and indistinguishable, and signs of regression were noted by Day 16 (1).

Changes in progestin level for Days 6-9 can not be evaluated in Table 1. The CL of the cow at six days was probably precocious in size, though the concentration and total progestins present fell within the four- to five-day range. Likewise, one cow at Day 9 showed levels within the ten- to 11-day range. Considering all cows from ten to 16 days, it appears that the CL reaches near maximum size by the tenth day, but that progestin concentration and total present are consistently higher at 14-16 days. This latter observation is not inconsistent with histological observations previously described (1).

Though bred and unbred cows are separately shown in Table 1, there is evidence that this separation is unnecessary. Foley and Greenstein (3), using

TABLE 1

Average levels of progesterone and 20 β-ol and the total of these two progestins in cows at intervals after estrus

After estrus or breeding	Cows	Corpus luteum						Ovaries[a]		Adrenals[a]	
		Wt.	Prog.	20 β-ol	Total	Total	Range	Wt.	Progestins	Wt.	Progestins
(days)	*(No.)*	*(g.)*	*(μg/g)*			*(μg.)*		*(g.)*	*(μg/g)*	*(g.)*	*(μg/g)*
					Unbred cows						
1	1	0	.....	.....	.....	.....	.....	13.9	1.8	19.7	<0.8
1	3	1.8[b]	3.9	1.8	5.7	10	0–24	14.7[3]	<1.7	26.9	2.3
2–3	3	1.1	46.9	22.2	69.1	76	7–197	14.5[3]	<2.6	27.4	1.3
4–5	3	2.0	22.6	5.9	28.5	57	14–117	17.5[3]	<1.7	24.5	<0.8
6	1	5.3	13.8	1.6	15.4	82	82	24.0	2.7	30.7	1.0
9	1	6.5	26.3	8.8	35.1	228	228	11.7	Lost	23.6	<1.8
10–11	3	6.8	22.4	4.4	26.8	182	106–252	15.8[3]	<1.4	21.6	<0.9
18–19	2	3.6	22.3	11.6	33.9	122	99–144	12.2[2]	<3.8	14.6	<1.0
22–24	3	6.4	7.4	3.3	10.7	68	15–94	15.2[3]	<1.2	24.0	<1.0
30–35	4	4.2	23.6	4.6	28.2	110	40–180	14.9[3]	4.9	19.5	<1.6
					Bred but not pregnant						
12–13	4	5.6	16.8	9.8	26.6	149	81–198	22.7[3]	<0.8	36.4	<0.4
14–15	3	7.4	25.9	13.1	39.0	289	175–386	18.7[3]	<1.7	23.7	<0.7
16	3	5.1	19.9	21.8	41.7	213	171–232	.....	....	.....	....
18–20	3	6.9	18.1	10.4	28.5	184	148–218	12.3[2]	2.0	20.7	1.6
					Pregnant						
16	1	5.5	21.8	5.6	27.4	151	151	12.1	<2.1	23.0	<0.6
20–21	2	5.4	18.8	23.5	42.3	228	180–282	.....	....	.....	....
25–34	7	6.1	15.3	11.1	26.4	161	58–553[c]	17.3[3]	<1.8	19.3	<1.3
37–42	6	6.2	27.1	13.2	40.3	250	115–465[d]	18.5[5]	<2.6	30.2[e]	<0.6

[a] Some quantities below sensitivity level of the method. Weight of ovaries does not include CL.
[b] Corpus albicans—preovulation.
[c] The next highest CL was 176.
[d] The next highest CL was 287.
[e] Range was 19 to 56 g.
[2, 3, 5]—Number of samples in the average for ovaries and adrenals.

CL from cows in early pregnancy, classified the various lutein cells into five types. Their Type I (immature) and Type II (mature) cells are considered functional and predominate in the normal pregnant cow from 16-28 days. The histological picture appears similar at 16 days in the nonpregnant (1) and pregnant cow (3). Loy *et al.* (9) found a significant correlation between functional cells (3) and progesterone concentration in the 14-day CL of heifers given progesterone the first day of the cycle, but the control correlation was −0.07. Foote *et al.* (5), using 14-day CL from normal and repeat breeder heifers, similarly observed a significant correlation of 0.57. Previous studies have shown that progesterone content of 14-day CL averages between 30-35 μg/g for normal (5, 16) and repeat breeder heifers (5) and pregnant heifers (16). When 20 β-ol is added to progesterone in 14-day pregnant animals, the average is 41.1 μg/g of CL (16). This level is almost identical to the averages in Table 1 for 14-16 day CL of cows bred at the previous estrus but not pregnant at slaughter.

After 16 days, progesterone concentration generally declined, even though the CL did not decline in weight. This decline in concentration appeared most rapid between 22-24 days. Though the CL of the three cows in this group were large, the progestin content was roughly one-fourth the earlier level. These results indicate that the CL regresses very rapidly in size just prior to estrus and that reduction in progestins precedes this by 24-28 hr. Edgar and Ronaldson (2) found that progesterone in ovarian vein blood of the cycling ewe reached near-maximum levels by Day 8 and remained high until about one day before the next estrus.

Progesterone level is closely associated with follicle growth, particularly as it relates to mitotic cell division of the thecal and granulosa layers. Cupps *et al.* (1) describes the first growth phase of the follicle as taking place during the third to fifth day of the cycle, an event coinciding with the lowest quantities of progesterone in the developing CL of the cow (Table 1) or the ovarian vein blood of the ewe (2). The high progestin content at mid-cycle (Table 1) compares with little or no mitotic activity in the two cell layers of the follicle. The second growth phase, primarily cell hypertrophy and infiltration of mast cells and eosinophils into the ovary, is most rapid during late proestrus and estrus (1). Table 1 shows that progestins in the normally aging CL are lowest during this period and suggests that progestin levels of about 100 μg. are associated with absence of heat and inhibition of follicle development at least beyond 10 to 15 mm. in size.

Early pregnancy. The data for early pregnancy (Table 1) indicate that high levels of progestins in the CL are maintained for at least 42 days. The range in concentration of progestins was from 19 to 67 μg/g of CL from 16 to 42 days of pregnancy. Omitting the highest and lowest values reduces the range from 22 to 47 μg/g. From 45 to 85 days the results were much more variable. Thirteen cows ranged from 7 to 71 μg/g, five were below 20 μg/g, and four were above 50 μg/g. (14). There is also evidence (Table 1) that progestin concentrations are lower at 25-34 days of pregnancy than at 37-42 days ($P < 0.10$).

However, total quantities present are not significantly different at $P < 0.10$. The 25-34 day levels agree well with the average of 26 μg/g observed at 28 days of pregnancy by Zimbelman *et al.* (16). At 42 days the two groups of data are not in agreement, being 19 μg/g for 42 days of pregnancy (16) and 40.3 μg/g for 37-42 days (Table 1).

Ovaries and adrenals. Thirty-one pairs of ovaries of nonpregnant cows averaged 16.1 g., which is very little different from the average of 15.6 g. reported previously for 54 pairs of ovaries from pregnant cows (14). Neither was there any difference in size of adrenals, averaging 24.4 g. for nonpregnant cows and 24.2 for pregnant cows (14). As previously noted for pregnant cows (14), the ovaries and adrenals of nonpregnant cows have relatively low quantities of progestins.

The adrenals from six cows (one to three days of the cycle) and two at 18-20 days all had sufficient quantities of progestins for accurate quantitation. The concentration was about twice the amount of progestins present at other times. Data like these only suggest that the bovine adrenal may be a significant source of progesterone, particularly when quantities in the ovary are low. One cow on Day 1 of the cycle had adrenals weighing 29 g., containing 90 μg. of progesterone. The Day 3 cow mentioned earlier, with only 7 μg. of progestins in the CL, had 21 μg. in the ovaries and 65 μg. in 38 g. of adrenals.

Post-partum. Results for four cows are shown in Table 2. No CL were present in the ovaries of three and in none of these was it possible to measure the progestins accurately. The adrenals of three were considerably above average in weight and all contained a considerable quantity of progestins, the highest being Cow 4 with 117 μg. The results suggest the need for closer scrutiny of the adrenal at this time as a possible reason for anestrus in the early post-partum cow.

Abnormal cows. Data for 20 cows, distinctly abnormal reproductively, are shown in Table 2. The five cows classified as repeat breeders were placed in this category because no gross abnormalities were observed at slaughter and none was bred at the estrus subsequent to slaughter. The ratio of 20 β-ol to progesterone was quite variable and shows no consistent pattern in this group of animals. The concentrations of progestins in the CL, ovaries, and adrenals were not unlike those shown for cows in a similar stage of the cycle in Table 1. The results are in general agreement with Foote *et al.* (5), in that they observed no difference in the progesterone levels of normal and repeat breeder heifers.

Two other repeat breeders (10 and 11) had abnormal embryos. In Cow 10, the embryo was quite degenerate, and though the corpus luteum weighed 3.0 g., it contained only 8 μg. of progestins. Cow 11 was carrying twins at 42 days, one of which was degenerating. This cow had an above-average level of progestins in the CL and the remaining portions of the ovaries. The third cow, 12, had conceived on one service, but probably would have aborted in a few days had she not been slaughtered. This cow at 118 days had a small CL (3.6 g.) but a high concentration of progestins per gram. The total quantity present was, however, no higher than in other cows in a similar stage of pregnancy (14).

TABLE 2

TABLE 2

Levels of progesterone and 20 β-ol and the total progestins in post-partum and reproductively abnormal cows

After calving or estrus	Cow	Corpus luteum				Ovaries[a]			Adrenals[a]		Remarks
		Wt.	Prog.	20 β-ol	Total	Total	Wt.	Prog-estins	Wt.	Prog-estins	
(days)	*(No.)*	*(g.)*	*(μg/g)*			*(μg.)*	*(g.)*	*(μg/g)*	*(g.)*	*(μg/g)*	
						Postpartum					
8[b]	1	2.8	0	Trace	.....	.....	8.2	Trace	37.7	0.5	
12[b]	2	None					16.9	<2.6	53.1	1.6	Post-partum paralysis
13[b]	3	None					16.5	<1.3	22.8	1.6	
22[b]	4	None					24.0	<2.2	58.4	2.0	Reproductive tract inflamed
						Repeat breeders					
11	5	5.5	18.4	0.7	19.1	105	18.2	0.9	22.8	<0.5	15 Services—mummified fetus
13	6	6.6	9.4	23.8	33.2	219	.....	....	.....		13 Services
13	7[c]	6.6	17.9	4.5	22.4	148	12.3	<3.4	14.3	3.4	8 Services
14	8[c]	8.4	19.3	26.7	46.0	386	19.5	<2.0	19.5	....	11 Services
20	9[c]	5.0	47.3	15.2	62.5	312	17.6	1.2	25.8	<0.7	14 Services; aborted at 6 mo.
						Abnormal embryo					
20	10[c]	3.0	1.3	1.3	2.6	8	12.3	<2.9	13.5	<1.4	20 Services
42	11	6.3	25.7	24.7	50.4	318	20.8	2.5	28.5	lost	6 Services; twins—1 dead
118	12	3.6	32.5	9.1	41.6	150	.....		.....		Degenerate placentomes
						Infected tract					
6	13[c]	2.2	7.4	1.8	9.2	20	9.7	2.6	14.3	<0.8	9 Services
15	14	10.8	10.6	15.9	26.5	286	22.7	<0.9	27.4	1.2	1 Service
76	15	4.8	8.1	4.6	12.7	61	25.2	4.6	43.9	8.3	Previous history—cystic
						Follicular cyst(s)					
4	16	5.5	20.5	5.6	26.1	144	33.7	<1.9	44.2	1.1	3 CL—one new
		23[d]	1.6[e]	0	1.6[e]	37					
9	17	1.5	12.3	8.6	20.9	31	13.4	<1.2	23.5	1.0	
		7[d]	<1.0[e]	<1.0[e]	<2.0[e]	<14					
17	18[c]	0	.....	.....	.....	.....	23.6[f]	12.9	24.6	<0.8	11 Services
30	19	0	.....	.....	.....	.....	35.8	<0.9	41.0	0.6	Fluid lost
58	20	3.2	25.1	7.6	32.7	105	34.1	3.9	20.4	<1.1	
						Luteal cyst(s)					
32	21	25[d]	3.0[e]	0.4[e]	3.4[e]	85					Ovaries not analyzed
36	22	4.4	36.2	10.0	46.2	203	6.1	4.2	.....		Left ovary by surgery
		10[d]	5.6[e]	1.6[e]	7.2[e]	72					
93	23	8.6	21.9	1.9	23.8	205	25.3	<1.0	20.6	<0.7	
		7[d]	1.1[e]	0.8[e]	1.9[e]	13					
55	24	7.4	26.2	28.2	54.4	403	16.1	<1.3			Right ovary by surgery
228	24	4.7	21.7	3.7	25.4	119	15.2	3.8	26.2	<3.6	

[a] Some quantities below sensitivity level of the method. Weight of ovaries does not include CL.
[b] Days since calving.
[c] Heifer.
[d] Milliliters fluid.
[e] Micrograms per milliliter.
[f] No organized CL was present in the ovaries.

None of the five cows with follicular-type cysts could be classified as a nymphomaniac, though all showed some gross symptoms of low-grade estrus. Two samples of follicular fluid each showed traces of progestins, but the quantity was not as high as the levels found in luteal cyst fluid. Heifer 18 is a special case, in that she had no organized luteal tissue but a high level of progestins in the ovaries (12.6 μg/g, or a total of 304 μg.).

The concentration of progestins in the three cows with luteal cyst fluid was on an average considerably lower than for the solid portions of the luteal cysts. Cow 16 was perhaps an unusual case, though some described by McEntee (10) appear generally similar. This cow had an apparently normal estrus four days before slaughter. One follicle had ruptured and a normal-appearing CL was forming. Another follicle on the same ovary was large and contained 23 ml. of fluid. Two other darkened CL were on the ovary. The three CL combined weighed 5.5 g. This ovary after fluid and CL removal weighed 19 g. The occurrence of large luteal cysts in cattle has been described (7, 10) and these may form in ovulatory or anovulatory follicles.

Exogenous progesterone has variable effects on the CL, depending on quantity administered, time of cycle or stage of early pregnancy, duration of treatment, and species (4, 9, 13, 17). As reviewed by Loy *et al.* (9), progesterone may prevent heat and ovulation without interfering with follicular development. There is evidence that estrogen is associated with greater luteinizing hormone (LH) activity and that this activity in swine is depressed by progesterone (4). Species differences may be considerable. Whether the degree of LH activity results from a production or release mechanism is unknown, though appreciable amounts of LH are found in the cow pituitary at all stages of the cycle (1). The quantity of LH in the pituitary varies significantly between days of the cycle, with levels being generally higher on the third and eighth days of the cycle (1). One postulation is that LH is more deficient or its release more inhibited when cystic follicles develop than when luteal cysts develop (10). If this postulation is true, then the two types of cysts could develop from a common defect (10). Levels of progestins in the CL and fluid from follicle-type cysts are generally lower than for the luteal types (Table 2). These observations, combined with observations in Table 1, suggest that optimum ratios of estrogen and progestins may be more important than specific levels of either type of hormone (9).

DISCUSSION

Progestins are essential for maintenance of pregnancy and, as earlier reviewed (6, 14), the principal source of the progestins appears to vary among species. Progesterone therapy during early pregnancy, in attempts to improve embryo maintenance, has resulted in conflicting results. Exogenous progesterone apparently has little or no effect on the pregnant rat (12) or ewe (17), beneficial effects in some cows (8) and humans (11), and deleterious effects in swine (12, 13).

In the present study, individual cow variation was a dominating feature of the data. The results suggest that progestin content of the corpus luteum has a

wide normal range and is either influenced by levels of other hormones or by the mechanisms which govern the efficiency of progestin release. After estrus but prior to ovulation, progestins are comparatively very low and this decline apparently occurs during the preceding two to three days. The difficulty of interpreting levels of hormone in glands which produce them, in terms of physiological significance, is recognized. But as reviewed earlier in the paper, the observations are not inconsistent with histological evidence (1, 3) and more direct comparisons (2, 5, 9, 13).

Though concentrations of progesterone and 20 β-ol are separately shown for CL in Tables 1 and 2, discussion was purposely limited to their combined values. Both show biological activity and 20 β-ol is presumably a metabolite of progesterone. Therefore, 20 β-ol may be higher or lower, depending on variable time between slaughter and storage in a deep frozen state. No significant differences in ratios were observed for pregnant cows and previous work indicates that 20 to 40% of the progestins in CL is 20 β-ol (6, 14). The average was 68% progesterone for CL from 89 pregnant cows (14), 75% for unbred cycling cows, and 60% for the bred but nonpregnant cows (Table 1).

ACKNOWLEDGMENT

The authors are indebted to Dr. K. R. Johnson (University of Idaho), Dr. H. T. Gier, and Dr. G. B. Marion (Kansas State University) for supplying part of the tissues used for this study, and to Miss Joan Dixon and Mrs. Kathryn Kanouse Farr for routine assay of the samples.

REFERENCES

(1) Cupps, P. T., Laben, R. C., and Mead, S. W. Histology of Pituitary, Adrenal, and Reproductive Organs in Normal Cattle and Cattle with Lowered Reproductive Efficiency. Hilgardia, 29: 383. 1959.

(2) Edgar, D. G., and Ronaldson, J. W. Blood Levels of Progesterone in the Ewe. J. Endocrinol., 16: 378. 1957.

(3) Foley, R. C., and Greenstein, J. S. Cytological Changes in the Bovine Corpus Luteum During Early Pregnancy. Proc. 3rd Sympos. Reproduction and Infertility, p. 88. Pergamon Press Ltd., London. 1958.

(4) Foote, W. C., Waldorf, D. P., Self, H. L., and Casida, L. E. Some Effects of Progesterone and Estradiol on the Ovarian Structures and on the Gonadotrophic Potency of the Pituitary Gland of the Gilt. J. Animal Sci., 17: 534. 1958.

(5) Foote, W. D., Zimbleman, R. G., Loy, R. G., and Casida, L. E. Endocrine Activity of Corpora Lutea from First Service and Repeat-Breeder Dairy Heifers. J. Dairy Sci., 42: 1944. 1959.

(6) Gorski, J., Erb, R. E., Dickson, W. M., and Butler, H. C. Sources of Progestins in the Pregnant Cow. J. Dairy Sci., 41: 1380. 1958.

(7) Johnson, K. R. Observations on Large Luteal Type Ovarian Cysts in a Herd of Dairy Cattle. J. Dairy Sci., 43: 863. 1960.

(8) Johnson, K. R., Ross, R. H., and Fourt, D. L. Effect of Progesterone Administration on Reproductive Efficiency. J. Animal Sci., 17: 386. 1958.

(9) Loy, R. G., Zimbleman, R. G., and Casida, L. E. Effects of Injected Ovarian Hormones on the Corpus Luteum of the Estrual Cycle in Cattle. J. Animal Sci., 19: 175. 1960.

(10) McEntee, K. Cystic Corpora Lutea in Cattle. Intern. J. Fertility, 3: 120. 1958.

(11) REIFENSTEIN, E. C., JR. The Maintenance of Human Pregnancy with Progestational Compounds. Proc. 3rd Sympos. Reproduction and Infertility, p. 88. Pergamon Press Ltd., London. 1958.

(12) SAMMELWITZ, P. H., DZUIK, P. J., AND NALBANDOV, A. V. Effects of Progesterone on Embryonal Mortality of Rats and Swine. (Abstr.). J. Animal Sci., 15: 1211. 1956.

(13) SPIES, H. G., ZIMMERMAN, D. R., SELF, H. L., AND CASIDA, L. E. The Effect of Exogenous Progesterone on Formation and Maintenance of the Corpora Lutea and on Early Embryo Survival in Pregnant Swine. J. Animal Sci., 18: 163. 1959.

(14) STORMSHAK, F., AND ERB, R. E. Progestins in Bovine Corpora Lutea, Ovaries, and Adrenals During Pregnancy. J. Dairy Sci., 44: 310. 1961.

(15) STORMSHAK, F., HUNT, MARJORIE L., AND ERB, R. E. Quantitative Methods for Determining Progestins in Corpora Lutea, Ovaries, and Adrenals of the Cow and Sow Using Progesterone-4-C^{14} as a Measure of Recovery of Extracted Hormone. J. Dairy Sci., 44: 300. 1961.

(16) ZIMBLEMAN, R. G., LOY, R. G., AND CASIDA, L. E. Biochemical Studies of the Bovine Corpus Luteum of Early Pregnancy. (Abstr.). J. Animal Sci., 18: 1551. 1959.

(17) ZIMBLEMAN, R. G., POPE, A. L., AND CASIDA, L. E. Effect of Exogenous Progesterone on the Corpus Luteum of the Bred Ewe. J. Animal Sci., 18: 1327. 1959.

VARIATION IN ESTRUAL CYCLES OF HOLSTEIN-FRIESIAN CATTLE [1]

S. E. MARES,[2] A. C. MENGE,[2] W. J. TYLER, AND L. E. CASIDA
Departments of Genetics and Dairy Husbandry, University of Wisconsin
and Dairy Cattle Research Branch, USDA, Madison, Wisconsin

SUMMARY

The lengths of intervals between estrual periods were studied in a herd which consisted of six sire lines of breeding and in which four systems of mating were practiced. The distribution and mean lengths of 766 estrual cycles and 191 return intervals from 221 heifers differed significantly ($P < 0.05$). The mean lengths were 22.3 days for estrual cycles and 25.3 days for return intervals. A repeatability estimate of estrual-cycle lengths was calculated for the heifers in each sire line and system of mating. Repeatabilities ranged from zero to 0.29 for the sire lines and from 0.02 to 0.21 for the systems of mating.

An analysis of variance showed estrual-cycle length in heifers to be affected significantly by the season of birth and the system of mating. The summer- and spring-born heifers had the longest cycles, 25.0 and 25.2 days, whereas the winter- and fall-born had cycles averaging 22.2 and 20.9 days, respectively. Daughters of inbred dams had the longest mean cycle lengths, 24.9 days for the outbred daughters and 25.6 days for the inbred; daughters of outbred dams averaged 20.6 for those that were outbred and 22.2 for those that were inbred.

An analysis of variance of the first estrual-cycle length following calving in outbred and inbred cows showed a significant interaction of sire line and system of mating. Repeatability estimates of the first estrual-cycle length following calving from one calving interval to another of 0.11 ($P < 0.10$) for the outbreds and 0.06 for the inbreds were calculated.

It is generally accepted that estrus occurs quite regularly throughout the year in the cow and that the modal length of the estrual cycle is 21 days. The mean length shows considerable variation; much of this variation may be due to differences in procedures in obtaining the data, but various tangible factors also may have an influence on the variation.

Effects of feed level, season, year, age, infertile service, and reproductive abnormalities on the estrual-cycle length in cattle have been studied by various workers (1, 2, 5-9, 12, 14, 16, 17). Studies on genetic variation in estrual-cycle length of cattle, with the exception of breed and individuality, have been lacking.

This study is an attempt to determine possible effects of sire line and system of mating on estrual-cycle length in a herd maintained for experimental studies.

Received for publication November 17, 1960.

[1] From the Department of Genetics (Paper No. 816) and Department of Dairy Husbandry; published with the approval of the director of the Agricultural Experiment Station. This study was supported in part by a grant from the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation and by a grant from the Emmons Blaine, Jr., Fund. Contribution from the Wisconsin Agricultural Experiment Station as a collaborator under the North Central Region Cooperative Research Project entitled Improvement of Dairy Cattle Through Breeding—NC-2, conducted in cooperation with the Dairy Cattle Research Branch, USDA.

[2] Agents of the Dairy Cattle Research Branch, USDA.

MATERIALS AND METHODS

The data for this study were collected from the Emmons Blaine, Jr., Holstein-Friesian Herd at Lake Mills, Wisconsin. The estrual cycles included in this report were observed during the period from December 21, 1949, to December 20, 1955. Hawk *et al.* (9) made a comparison of the estrual cycles and the return intervals which occurred between January 1, 1950, and July 31, 1953, in this same herd. The herd consists of six unrelated sire lines with four systems of mating, as was outlined in that report.

A sire line includes all the descendants (through the male parent) of a particular foundation sire and the symbols used to designate the system of mating are as follows: O-O, outbred animal from an outbred dam; O-I, outbred animal from an inbred dam; I-O, inbred animal from an outbred dam; and I-I, inbred animal from an inbred dam. Sire lines were considered to be representative of the Holstein population and treated as a random variable in the statistical analyses, while system of mating was treated as a fixed variable. The data were analyzed separately for heifers and parous cows.

Heifers representing all four systems of mating were studied, whereas cows from only the O-O and I-O systems were included because of small numbers in the other two. The average inbreeding coefficient was 22% in the I-O system of mating for both heifers and cows and was 33% in the I-I system of mating for the heifers. In addition, outbred control heifers (37 in number) obtained by mating O-O dams to nonproject sires in various artificial breeding studs contributed data for a portion of the study.

The management practices followed in this herd for maintaining environment as constant as possible were described by Buch *et al.* (3), and the standard procedures for culling cows from the herd were described by Buch *et al.* (4). All animals over 6 mo. of age were checked for estrus twice daily for 15 to 30 min. at approximately 12-hr. intervals. An individual was considered to be in estrus if mounting by other females was allowed.

The data were examined in the following steps: (1) the distribution of estrual-cycle and return-interval lengths in heifers, (2) the repeatability of estrual-cycle and return-interval lengths in heifers, (3) the effects of season of birth, sire line, and system of mating on estrual-cycle length in heifers, (4) the effect of sire line and system of mating on estrual-cycle length in parous cows, and (5) the repeatability of length of the first estrual cycle following calving from one calving interval to another in parous cows.

The study of repeatability of estrual cycles in heifers included all of the cycles occurring from the time an animal became 1 yr. of age until the first insemination. This insemination was at the first estrus on or after 15 mo. of age, provided the animal's reproductive organs were in a satisfactory breeding condition as determined by rectal palpation. The interval between the estrus of insemination and the return to estrus, if the animal did return, is considered as a return interval. Only those return intervals in which no evidence of conception could be found, i.e., presence of uterine contents including palpable

membranous material at 35-41 days post-insemination or returning to estrus before this time, were included in the study.

After transforming estrual-cycle and return-interval lengths to logarithms to normalize their distributions, an estimate of repeatability using the method of intraclass correlation (15) was obtained. The repeatability of estrual-cycle lengths was calculated for each sire line and system of mating, whereas an overall repeatability was calculated for return-interval lengths due to the small numbers of return intervals in the groups. The variances of estrual-cycle length within heifer were tested for heterogeneity among sire lines and systems of mating.

An analysis of variance due to season of birth, sire line, and system of mating was made by the method of fitting constants (10). Only the two cycle lengths of each heifer immediately preceding insemination were used. A mean of these two cycles was transformed to a logarithm and the analysis made on these transformed means. The season of birth refers to the season in which the heifer was born: winter, December 21-March 20; spring, March 21-June 20; summer, June 21-September 20; fall, September 21-December 20.

An analysis of variance of estrual-cycle length in cows was made by the method of unweighted means (15), using only the first cycle following a normal calving in each parity. This cycle length was adjusted to remove any effect of season of calving and transformed to a logarithmic value for analysis. Repeatability of estrual-cycle length from one calving interval to the next, using only the first cycle of each parity, was also calculated. Parities in which cystic ovaries, retained placenta, or stillbirths occurred were not included.

RESULTS AND DISCUSSION

Distribution of estrual-cycle and return-interval lengths. A total of 766 estrual cycles from the 221 heifers (including 37 control heifers) had an arithmetic mean length of 22.3 days and a range of three to 102 days. These same animals had a total of 191 return intervals, with a mean of 25.3 days and a range of four to 92 days. The mean lengths for estrual cycles and return intervals, using transformed data, were 20.1 and 22.3 days, respectively, and were found to differ significantly ($P < 0.05$). The modal length for the estrual cycles was 21 days and the number of cycles in this group represented 18.4% of the total (Table 1). The corresponding values for the return intervals were 20 days and 13.1% of the total.

The means found for the untransformed data are similar to the 24.1 and 26.4 days for estrual cycles and return intervals reported by Hawk *et al.* (9), which were for the same herd, but included cycles of cows in addition to those of heifers. They are considerably lower than the 30.8 days for estrual cycles following no service and 34.9 days for cycles following service reported by Chapman and Casida (5), as well as those reported by Erb *et al.* (6), which were 29.7 and 38.0 days, respectively.

These differences are attributed to the closer observation for signs of estrus in this herd than in most herds. The fact that many pregnancies lost at an

TABLE 1
Distribution of estrual-cycle and return-interval lengths

Length (days)	Estrual cycles		Return intervals	
	No.	%	No.	%
<18	97	12.7	24	12.6
18–23	541	70.6	106	55.5
24–28	30	3.9	15	7.8
29–33	12	1.6	11	5.8
34–38	16	2.1	5	2.6
39–43	41	5.3	12	6.3
>43	29	3.8	18	9.4
	766	100.0	191	100.0

$X^2 = 31.7$; $P < 0.01$.

Within 18-23 day range

Length (days)	Estrual cycles		Return intervals	
	No.	%	No.	%
18	51	6.7	21	11.0
19	106	13.8	20	10.5
20	121	15.8	25	13.1
21	141	18.4	15	7.8
22	89	11.6	19	10.0
23	33	4.3	6	3.1
	541	70.6	106	55.5

$X^2 = 31.7$; $P < 0.05$.

early stage, which ordinarily go unnoticed, were eliminated from these return intervals also tends to lower the mean for return intervals. The effect of age (2, 5, 6) may also contribute to the greater means found in the other studies, since they included cycles of several parities for many of the cows.

Estrual-cycle and return-interval lengths were arbitrarily divided into the day groups, as shown in Table 1. The distributions of estrual-cycle and return-interval lengths were compared by a Chi-square test and found to differ significantly ($P < 0.01$). The percentages of estrual cycles and return intervals less than 18 days in length are about equal (12.7 vs. 12.6), but a total of 70.6% of the estrual cycles fell in the 18–23-day range as compared to 55.5% of the return intervals. Since the mode for estrual-cycle length (21 days) was one day longer than the mode for return-interval length (20 days), the distributions within the 18–23-day range were tested by Chi-square. They were found to differ significantly ($P < 0.05$). The 24–33-day range contains a considerably higher percentage of return intervals (13.6%) as compared to estrual cycles (5.5%). This would seem to indicate a delay of return to estrus following inseminations. Whether insemination itself affects cycle length in cattle is doubtful. Marion *et al.* (11), using a vasectomized bull, found that the act of mating did not lengthen the subsequent cycle in heifers representing four dairy breeds. As suggested by Hawk *et al.* (9), the average delay of returning to estrus following insemination may be due to the incidence of early embryonic death.

Another possible explanation is based on the assumption of a correlation between certain factors causing potential failure in fertilization and greater lengths of subsequent cycles. A larger proportion of longer-than-normal intervals would then be represented in return intervals than in estrual cycles.

The 34–43-day range contains about equal percentages of estrual cycles and return intervals (7.4 vs. 8.9), whereas the percentage of return intervals (9.4) over 43 days in length again exceeds the percentage of estrual cycles (3.8).

TABLE 2

Repeatability of estrual cycle length
(intra-heifer correlations)

System	Line						Within lines for each system
	1	2	3	4	5	6	
O-O	.11	.22†	.05	.00	.01	.01	.03
O-I	.43†	....	.00	.00	.15	.33†	.21*
I-O	.04	.54**	.00	.14	.22*	.37*	.17**
I-I	.00	.49†	.01	....	.00	.03	.02
Within systems for each line	.05	.29**	.00	.00	.10	.20*	

* $P < 0.05$.
** $P < 0.01$.
.... No observation.
† $P < 0.10$.

The repeatability of estrual-cycle and return-interval lengths in heifers. The repeatability of estrual-cycle length for each sire line and system of mating is presented in Table 2. Two of the lines (2 and 6) and two of the systems of mating (O-I and I-O) show a significantly greater variation in cycle length between heifers than within heifers. In testing for heterogeneity, the within-heifer variances among lines were found to differ significantly ($P < 0.05$), whereas the within-heifer variances among systems of mating did not differ significantly. It would appear that heifers of some lines were affected more by temporary environmental factors, external and internal, than heifers of other lines. The system of mating, however, did not affect this sensitivity. Significant repeatabilities as high as 0.29 for one line and 0.21 for one system indicate that heifer differences may be appreciable under certain conditions. A significant repeatability ($P < 0.05$) for the various line-system-of-mating groups was found only in the I-O system of mating in three of the lines (2, 5, and 6). Repeatabilities which approached significance ($P < 0.10$) were found in the O-O system of Line 2, the O-I system of Lines 1 and 6, and the I-I system of Line 2. Lines 3 and 4 had nonsignificant repeatabilities in all of the systems of mating represented. From this, it appears that sire line interacts with the system of mating to affect the repeatability of estrual-cycle length.

Pou *et al.* (13) reported a repeatability for estrual-cycle length of 0.18. Olds and Seath (12) reported values of 0.07 and 0.08 from a study of cycles including service cycles.

TABLE 3

Analysis of variance of estrual-cycle length of heifers by method of fitting constants

Source	D.F.	Error term	M.S.
Season of birth	3	R	.045*
Sire line (L)	5	W	.014
System of mating (S)	3	L × S	.050**
L × S	15	W	.007
Residual interaction (R)	44		.016*
Within (W)	100		.022

* Composed of the mean squares of the possible interactions involving season.
* $P < 0.05$.
** $P < 0.01$.

A total of 105 return intervals representing 33 heifers, having two or more return intervals, was used to obtain an estimate of repeatability for return-interval length. The variation in return-interval length between heifers did not differ significantly from the variation within heifers giving a repeatability of 0.06.

The effects of season of birth and system of mating on estrual-cycle length in heifers. A total of 171 heifers exhibiting two or more estrual cycles while between 12 and 15 mo. of age contributed data for a study of these effects. An analysis of variance (Table 3) showed a significant effect of season of birth ($P < 0.05$) and system of mating ($P < 0.01$). Table 4 shows the mean lengths of estrual cycles and the numbers and percentages of estrual cycles whose lengths were less than 18 days, from 18 to 23 days, and more than 23 days for each season and system. The longer mean estrual-cycle length in the summer- and spring-born heifers appears to be due primarily to an increased number of long cycles. These animals had 27.9% of their cycles of more than 23 days in length, as compared to 8.3 and 14.4% for the fall- and winter-born heifers, respectively.

The significant effect of season of birth of a heifer on the length of her estrual cycle is not necessarily an effect of the season of birth itself or even of

TABLE 4

Effects of season of birth and system of mating on estrual-cycle length

	Estrual-cycle length (days)							
	< 18		18–23		> 23		Summary	
Season	No.	%	No.	%	No.	%	No.	Mean length
Fall	7	14.6	37	77.1	4	8.3	48	20.9
Winter	14	13.5	75	72.1	15	14.4	104	22.2
Summer	7	8.1	55	64.0	24	27.9	86	25.0
Spring	10	9.6	65	62.5	29	27.9	104	25.2
System								
O-O	24	19.4	84	67.7	16	12.9	124	20.6
I-O	13	9.8	88	66.7	31	23.5	132	22.2
O-I	1	2.6	26	68.4	11	29.0	38	24.9
I-I	0	0.0	34	70.8	14	29.2	48	25.6

the season when the cycles occurred. It may be due to some intermediate stage of development which would be correlated with season of birth, since these estrual cycles were from animals of a constant age (12-15 mo.).

The longer mean estrual-cycle length in the O-I and I-I heifers appears to be due primarily to an absence of short cycles in these animals. Only one cycle (2.6%) was less than 18 days in length, as compared to 19.4 and 9.8% for the O-O and I-O heifers, respectively. The O-O heifers also had fewer cycles more than 23 days in length than did the other three groups.

Since heifers from systems of mating in which the dam is inbred (O-I and I-I) have longer estrual-cycle lengths than heifers from systems of mating in which the dam is outbred (O-O and I-O), a maternal effect seems to be implicated. A theoretical physiological basis for this effect was not apparent, however, in the growth rates or ages at puberty of these animals.

The effect of sire line, system of mating, and individuality on estrual-cycle length in parous cows. A total of 346 first estrual cycles following calving from 205 cows was included in this study. An analysis of variance (Table 5) showed

TABLE 5

Analysis of variance of the first estrual-cycle length following calving in cows by method of unweighted means

Source	D.F.	M.S.
Sire line (L)	5	.070
System of mating (S)	1	.001
L × S	5	.104*
Between cows	193	.043

* $P < 0.05$.

a significant interaction ($P < 0.05$) of sire line and system of mating. Within the systems of mating (Table 6) the sire-line means had ranges of 4.5 days for the O-O cows and 11.8 days for the I-O cows. Within the sire lines, the system-of-mating means had ranges varying from 1.2 days (Line 6) to 6.8 days (Line 3).

TABLE 6

Mean lengths of the first estrual cycle following calving in cows

	Line							
System	1	2	3	4	5	6	Av.	Range
O-O	25.2	23.9	20.7	23.9	24.6	24.1	23.8	4.5
I-O	21.7	22.3	27.5	17.5	29.3	25.3	23.5	11.8
Av.	23.4	23.1	24.1	20.7	27.0	24.7	23.6	
Range	3.5	1.6	6.8	6.4	4.7	1.2		

A repeatability estimate from one calving interval to another of the first estrual-cycle length following calving was obtained on 90 cows having 231 cycles. The outbreds (O-O) had a repeatability of 0.11, which approached significance ($P < 0.10$), whereas the inbreds (I-O) had a nonsignificant re-

peatability of 0.06 which did not differ significantly from the value for the outbreds.

The I-O heifers had a significant repeatability of 0.17, which is considerably higher than the value of 0.06 found for the I-O cows. The O-O animals did not show such a difference, with repeatabilities of 0.03 and 0.11 for heifers and cows, respectively.

REFERENCES

(1) Anderson, J. The Periodicity and Duration of Oestrus in Zebu and Grade Cattle. J. Agr. Sci., 34: 57. 1944.

(2) Asdell, S. A., DeAlba, J., and Roberts, S. J. Studies on the Estrous Cycle of Dairy Cattle: Cycle Length, Size of Corpus Luteum, and Endometrial Changes. Cornell Vet., 39: 389. 1949.

(3) Buch, N. C., Tyler, W. J., and Casida, L. E. Postpartum Estrus and Involution of the Uterus in an Experimental Herd of Holstein-Friesian Cows. J. Dairy Sci., 38: 73. 1955.

(4) Buch, N. C., Tyler, W. J., and Casida, L. E. Variation in Some Factors Affecting the Length of Calving Intervals. J. Dairy Sci., 42: 298. 1959.

(5) Chapman, A. B., and Casida, L. E. Analysis of Variation in the Sexual Cycle and Some of Its Component Phases with Special Reference to Cattle. J. Agr. Research, 54: 417. 1937.

(6) Erb, R. E., Ehlers, M. H., and Morrison, R. A. Environmental Influences on Frequency of Estrous Cycles. Washington Agr. Expt. Sta., Bull. 583. 1958.

(7) Hall, J. G., Branton, C., and Stone, E. J. Estrus, Estrous Cycles, Ovulation Time, Time of Service, and Fertility of Dairy Cattle in Louisiana. J. Dairy Sci., 42: 1086. 1959.

(8) Hammond, J. J. The Physiology of Reproduction in the Cow. Cambridge University Press, London. 1927.

(9) Hawk, H. W., Tyler, W. J., and Casida, L. E. Effect of Sire and System of Mating on Estimated Embryonic Loss. J. Dairy Sci., 38: 420. 1955.

(10) Kempthorne, Oscar. The Design and Analysis of Experiments. John Wiley and Sons, Inc., New York. 1952.

(11) Marion, G. B., Smith, V. R., Wiley, T. E., and Barrett, G. R. The Effects of Sterile Copulation on Time of Ovulation in Dairy Heifers. J. Dairy Sci., 33: 885. 1950.

(12) Olds, D., and Seath, D. M. Repeatability of the Estrous Cycle Length in Dairy Cattle. J. Dairy Sci., 34: 626. 1951.

(13) Pou, J. W., Anderson, C. R., Asdell, S. A., Sykes, J. F., and Jones, R. C. A Study of the Inheritance of Breeding Efficiency in the Beltsville Dairy Herd. J. Dairy Sci., 36: 909. 1953.

(14) Roark, D. B., and Herman, H. A. Physiological and Histological Phenomena of the Bovine Estrual Cycle with Special Reference to Vaginal-Cervical Secretions. Missouri Agr. Expt. Sta., Bull. 455. 1950.

(15) Snedecor, G. W. Statistical Methods. 5th ed. Iowa State College Press, Ames, Iowa. 1956.

(16) Sorenson, A. M., Hansel, W., Hough, W. H., Armstrong, D. T., McEntee, K., and Bratton, R. W. Causes and Prevention of Reproductive Failure in Dairy Cattle. I. Influence of Underfeeding and Overfeeding on Growth and Development of Holstein Heifers. Cornell Univ. Agr. Expt. Sta., Bull. 936. 1959.

(17) Trimberger, G. W. Ovarian Functions, Intervals Between Estrus, and Conception Rates in Dairy Cattle. J. Dairy Sci., 39: 448. 1956.

EFFECT OF TOTAL DIGESTIBLE NUTRIENT INTAKE FROM BIRTH TO FOUR YEARS OF AGE ON GROWTH AND REPRODUCTIVE DEVELOPMENT AND PERFORMANCE OF DAIRY BULLS [1]

R. J. FLIPSE AND J. O. ALMQUIST

Dairy Breeding Research Center, Department of Dairy Science
The Pennsylvania State University, University Park

SUMMARY

Neonatal Holstein bull calves were assigned to four levels of energy intake representing 70, 100, 115, and 130% of the National Research Council recommended TDN allowances for growing dairy calves. The groups were designated L, N, M, and H, respectively. Two additional groups were on a changeover at 104 wk. of age—LH changed from 70 to 130% and HL from 130 to 70%. Data were collected on growth (body weight, height at withers, and heart girth), onset of semen production, and semen quantity and quality until the bulls reached 208 wk. of age. The average age at the onset of semen production was 61, 45, 41, and 44 wk., respectively, for the L, N, M, and H groups. Comparable figures for body weight at the onset of semen production were 523, 643, 675, and 784 lb.

At 80 wk. of age, the average motile sperm output per ejaculate was 1.15 billion for the L group; this was about 50% of that for the H group. After 112 wk. of age there was no difference in output of motile sperm per ejaculate associated with level of energy intake.

Between 156 and 208 wk. of age, the H bulls became much slower in sexual reaction time than bulls in other groups. During this period weaknesses of the feet and legs also became evident in the H bulls. Depletion trials at 4 yr. of age confirmed the relatively low sexual responses of the H bulls and also showed that M as well as H bulls were deficient in their ability to recover response after satiation to a stimulus animal. Sperm output and extra-gonadal sperm reserves were similar among bull groups.

Limited fertility data collected on the L, N, and H groups showed no marked difference in fertility attributable to the level of TDN intake.

Within the past decade much interest has centered on the effects of nutrition on reproductive performance of farm animals. A number of reviews on various aspects of this subject have appeared; those of Reid (19), Reece (18), and Casida (5) provide current appraisals of this area of investigation. Asdell *et al.* (1) have discussed a cooperative regional approach to the problem.

Much of the work with cattle has involved measuring the response to variations in the plane of nutrition, although the results commonly are attributed to variations in energy intake. The rationale for the implication of energy (19) has been based on the idea that only a limited number of specific nutrients are involved in reproduction; some of the nutrients which are involved influence appetite and, thus, the energy intake.

Received for publication November 25, 1960.

[1] Authorized for publication on November 16, 1960, as paper No. 2507 in the journal series of The Pennsylvania Agricultural Experiment Station.

Supported in part by funds from the regional project NE-1 entitled Causes and Prevention of Reproductive Failures in Dairy Cattle, a cooperative study involving Agricultural Experiment Stations in the Northeastern region and the Dairy Husbandry Research Branch, U. S. Department of Agriculture.

The present study was undertaken in 1950 to determine specifically the effects of variations in energy intake on the growth, reproductive development, and reproductive performance of Holstein bulls, whereas other nutrients were maintained at adequate and similar levels in all experimental groups. The last replications completed the 4-yr. experimental period in 1958.

EXPERIMENTAL PROCEDURE

Four levels of energy intake, expressed as total digestible nutrients, were tested as to their effects on growth, reproductive development, and subsequent reproductive performance of Holstein bulls. The control group (N) received the National Research Council recommended allowance for total digestible nutrients (TDN) (12). The low group (L) received 70% of this allowance, whereas medium (M) and high (H) groups received 115 and 130%, respectively. Protein and mineral intakes exceeded the recommended allowances for these nutrients and were similar in all groups. Composition of the rations and details of feeding and management have appeared in a preliminary report (8).

Space limitations prevented keeping all 24 experimental animals on trial for the full 4-yr. period of experimentation. Thus, as bulls reached 2 yr. (104 wk.) of age, random assignments (within each of the four main groups) were made as follows:

Experimental group	Change made at 104 wk. of age	Subgroup designation	No. of bulls
L	No change	L	3
	To H	LH	3
N	No change	N	3
	Removed from expt.	..	3
M	No change	M	3
	Removed from expt.	..	3
H	No change	H	3
	To L	HL	3

Where changes in feeding program were made (LH and HL subgroups), they were done gradually over the 8-wk. period of 105 to 112 wk. of age. Ration changes were made at 2 yr. of age, to ascertain what effects a specified level of energy would have on reproductive performance, and the extent to which early nutrition might influence subsequent performance on a different energy level.

Sexual development and semen production were evaluated as previously reported (8). In addition, starting at 3 yr. of age, sexual activity was measured using reaction time to ejaculation with a constant stimulus animal. However, in some cases it was necessary to introduce a new stimulus animal in order to achieve ejaculation. Sexual preparation consisted of 2 min. of restraint and one false mount before ejaculation.

Upon reaching 4 yr. of age, two 2-hr. depletion trials at a seven-day interval were conducted with each bull to gather data on sperm output, sperm reserves, and behavioral characteristics (9). During the first depletion trial, the bull

was permitted to ejaculate voluntarily with a constant-stimulus animal for 2 hr. or until satiated (no response in 30 min.). One week later, recovery of sexual response to the same stimulus animal was measured by comparing the number of ejaculates obtained during the first hour of the second trial with the number obtained during the first hour of the first trial. After 1 hr. with the initial animal, response to new stimulus animals was determined immediately by offering three new animals at 20-min. intervals. Extra-gonadal sperm reserves were calculated on the basis of sperm output, using the value reported by Hale and Almquist (9), that an average of 53.4% of the total sperm reserves is removed by adequate depletion.

RESULTS

Feed intake was carefully regulated throughout the experiment; each animal's ration was adjusted weekly on the basis of his body weight. Actual feed intake at 104 wk. was within 0.5% of the prescribed levels of 70, 100, 115, and 130% of the recommended allowance for TDN. From 2 to 4 yr. of age, feed consumption was maintained in all but the H group, when consumption declined slightly. During the 4 yr. of the trial the H group consumed an average of 128% of the recommended allowance.

The summarized data on body weight, height at withers, and heart girth are presented in Table 1. As would be expected, the greatest differences occurred during the period of most rapid growth. For example, at 48 wk. the L group

TABLE 1

Body weight, height at withers, and heart girth of Holstein bulls on various levels of TDN intake

(Mean of three bulls per group)

Age in weeks	Group					
	L	N	M	H	LH	HL
			Weight *(lb.)*			
0	96	95	102	105	102	96
48	428	669	819	831	395	843
96	876	1,276	1,510	1,557	847	1,635
112	1,003	1,438	1,708	1,721	1,077	1,762
160	1,309	1,776	2,185	2,164	1,757	1,844
208	1,462	1,945	2,424	2,488	2,117	1,869
			Height at withers *(cm.)*			
0	74	76	76	77	75	74
48	107	116	123	122	107	120
96	128	135	142	140	127	137
112	134	140	146	145	133	142
160	143	145	155	151	146	148
208	148	149	157	155	153	151
			Heart girth *(cm.)*			
0	81	81	84	84	81	81
48	132	157	168	165	135	168
96	170	198	206	208	173	211
112	178	203	213	216	185	216
160	197	216	232	235	220	219
208	203	226	244	246	236	221

averaged 63% of the body weight of the N group. At 96 wk. this figure had increased to 69%, and at 208 wk. to 75%. Similar to findings with beef cattle (22), bulls restricted in energy for the first 2 yr. (LH) recovered completely when fed liberally after 2 yr. of age. Growth performance for both the M and H groups was above the Morrison average (16), whereas the N group was slightly below this average.

The age, body weight, height at withers, and heart girth at the time the first ejaculate containing motile spermatozoa was collected are shown in Table 2.

TABLE 2

Age and measurements of Holstein bulls on various levels of TDN intake at the time the first ejaculate containing motile sperm was collected

Feeding level	No. bulls	First ejaculation			
		Age	Weight	Ht. at withers	Heart girth
		(wk.)	*(lb.)*	*(cm.)*	*(cm.)*
L	8	61 ± 4[a]	523 ± 40	114 ± 3	144 ± 4
N	8	45 ± 3	643 ± 41	114 ± 1	153 ± 3
M	6	41 ± 2	675 ± 42	117 ± 2	155 ± 4
H	8	44 ± 2	784 ± 41	119 ± 1	163 ± 3

[a] Mean and standard error.

The difference in age between the L and each of the other groups was significant at the 1% level of probability. The other statistically significant differences were: weight, L and H ($p \leqslant 0.01$); weight, L and M ($p \leqslant 0.05$), and heart girth, L and H ($p \leqslant 0.01$). As observed by Bratton *et al.* (4), the height at withers did not vary greatly with TDN intake.

Data on total motile sperm output per ejaculate are shown in Table 3. As previously noted (8), bulls on restricted energy intake produced poorer quality semen than did more liberally fed bulls early in the trial, but as the bulls became older this difference disappeared. The change from H to L at 2 yr. of age appeared to have an adverse effect on volume of semen; this is reflected in total motile sperm output (HL group, Table 3).

TABLE 3

Average output of motile sperm in billions per ejaculate of Holstein bulls on various levels of TDN intake
(Mean of three bulls per group)

Group	Age in weeks							
	57–80	81–104	105–112	113–136	137–160	161–184	185–208	113–208
L	1.01	3.24	4.56	4.35	4.88	4.71	5.62	4.76 ± 1.32[a]
N	1.10	2.45	2.67	2.96	3.62	3.72	3.85	3.65 ± 0.85
M	2.21	2.85	3.92	4.65	4.83	5.07	6.12	5.11 ± 1.85
H	2.30	3.94	3.85	4.66	4.62	4.09	5.17	4.56 ± 1.32
LH	0.93	1.23	1.96	3.07	3.61	4.43	4.99	4.00 ± 0.21
HL	2.26	3.51	3.27	3.78	3.41	4.40	4.99	4.14 ± 1.23

[a] Standard error.

Sexual behavior information measured by reaction time to ejaculation is shown in Figure 1. Collection of these data was initiated at 3 yr. of age, when it became obvious that the reaction time of the H bulls was increasing. Significant differences were noted in the time required for ejaculation, with a mean of 9.2 min. for H, 3.7 min. for M, and 1.5 min. or less for each of the other groups.

During the 4th yr. of the experiment, each of the bulls in the H group developed visual evidence of weakness of the feet and legs. Whether these weaknesses were coincidental to, or the partial cause of, lowered sexual activity ratings and increased time for ejaculation is subject to speculation.

The data obtained from the depletion trials, presented in Tables 4 and 5, confirm and extend those obtained by weekly semen collections and measures of sexual reaction time. In general, sperm output and reserves were similar for all groups. Although only one bull in the H group was sufficiently active to provide adequate depletion data, his sperm reserve was comparable to those

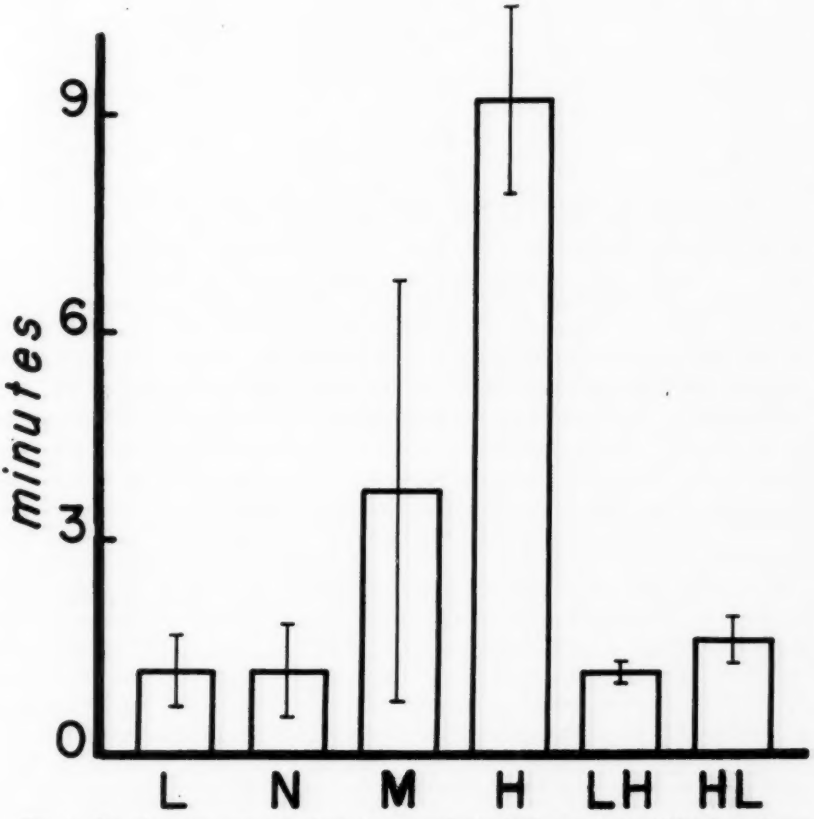


FIG. 1. Reaction time per ejaculate (mean and standard error) for Holstein bulls on various levels of TDN intake.

of bulls in other groups. Two of the LH bulls were slightly deficient in sexual activity in the first depletion and showed low sperm output, but they responded sufficiently in the second depletion to show that they were not lacking in sperm reserves. All bulls which possessed sufficient sexual activity to give adequate depletion measures in both the first and second trials, including two M bulls and one H bull, showed complete or essentially complete replenishment of sperm reserves in seven days. Therefore, there was no evidence that any of the bulls on the experiment were deficient in sperm production on the basis of 1 wk. of replenishment of depleted sperm reserves.

TABLE 4

Sperm output and reserves at 4 yr. of age as measured by depletion trials at a seven-day interval

Group	1st depletion		2nd depletion		Est. extragonadal sperm reserves in billions[a]
	Av. No. of ejac.	Av. No. of sperm in billions	Av. No. of ejac.	Av. No. of sperm in billions	
L	11	40.9	16	40.8	76.4
N[b]	17	39.7	25	37.0	71.8
M	8	30.5	11	36.0	75.1
H[c]	7	35.1	9	38.6	72.2
LH	8	26.7	14	35.8	66.9
HL	22	42.2	27	37.3	74.4

[a] Based on sperm output for adequate depletion in one or both trials.
[b] One bull excluded because of inadequate depletion in first trial.
[c] Represents only one bull, as other two were too inactive to be depleted.

The M and H groups showed distinctly inferior ability to recover sexual response to the same stimulus animal in 1 wk. (Table 5). The only bulls failing to respond well to the introduction of new stimulus animals were one H bull and one LH bull. Although wide variation in recovery was expected (9), none of the bulls in the M or H groups approached 100% recovery, whereas at least

TABLE 5

Recovery of sexual response after satiation and response to new-stimulus animals at 4 yr. of age

Group	Av. No. of ejac. 1st hr. of 1st depletion	Per cent recovery 1st. hr. of 1st depletion	Av. No. of ejac. in 1 hr. with three successive new S.A.[a]
L	8	74	9
N	8	74	14
M	7	47	10
H[b]	5	38	4
LH	7	80	7
HL	15	88	13

[a] Three new-stimulus animals (S.A.) presented in succession for 20-min. each after 1 hr. with initial test animal in second depletion.
[b] Represents two bulls; one bull not tested, as he failed to respond to a wide variety of stimuli before reaching 4 yr. of age.

one bull in each of the other groups showed 100% recovery. Nine of the 12 bulls in the groups other than M and H were higher in recovery than the highest bull in the M and H groups (57%). However, another H bull failed to respond to new-stimulus animals before reaching 4 yr. of age and could not be included in the depletion trials.

Data collected on the fertility phase of the experiment were meager, with only two bulls each on the L, N, and H groups, and only 8,124 first services to the six bulls in the 2 yr. immediately following their return to the breeding organization. Fertility data were as follows:

			% 60- to 90-day nonreturns	
Group	Bull	No. 1st services	Individual bulls	Group means, weighted by bulls
L	1	1,007	72.9	71.3
	2	1,560	69.7	
N	3	1,753	63.7	68.9
	4	867	74.0	
H	5	1,695	75.9	72.6
	6	1,242	69.2	

Although individual differences are evident, it appears that the levels of energy intake employed had no drastic effect on subsequent fertility.

DISCUSSION

While the results in this study have been attributed to differences in energy intake, a word of caution is in order, in that the nutritive ratio varied inversely with the energy intake. This was necessary in order to vary energy intake while maintaining equivalent intakes of protein and minerals. Whereas it is unlikely that nutritive ratios varying within the range employed (1:3.6 for L to 1:6.0 for H) would affect reproductive performance markedly, the possibility has not been excluded by controlled experimentation.

Ignoring minor discrepancies, which may or may not be due to differences in experimental design, several studies are in general agreement with regard to the relationship between plane of nutrition and onset of semen production (1, 4, 6, 10, 11, 13, 20). In general, low levels of feeding were observed to delay the onset of semen production 3 to 5 mo. in comparison to moderate and high levels of feeding. The fact that similar differences were observed between the N and L groups in the present study indicates that energy is the major limiting factor when intake is restricted. On the other hand, some investigations (4, 11) have indicated greater differences between normal and high planes of nutrition than were shown between normal and high levels of energy intake in the present

study. If such differences are actual rather than apparent, it is logical to attribute them to the increased protein, minerals, and/or vitamins supplied in the plane of nutrition studies. Unpublished data (7) indicate that quantity of protein has little or no effect on the onset of semen production in bulls, but the specific effects of supplements of most minerals and vitamins, as well as nutrient interactions, have not been determined.

With regard to sperm output, the adverse effects of low energy intake are obvious during the early weeks of semen production. In this study, at 80 wk. of age the average motile sperm output per ejaculate for the L group was about 50% of that for the H group. James (10) reported similar results, and the data of Bratton *et al.* (4) indicated that underfed bulls produced about 50% as many motile sperm per ejaculate as did overfed bulls at 74-80 wk. of age. However, if one examines total motile sperm output from 113 to 208 wk. (Table 3), little difference can be noted between high and low levels of energy intake. This supports the observation of Mann and Walton (14): severe underfeeding did not alter the sperm output of a mature bull. One the other hand, Olson (17) stated that with mature monozygous triplets, semen quality and quantity favored bulls receiving 100 and 130% TDN over those receiving 70% TDN.

In this study, a maximum of one ejaculate per bull per week was collected, using a low level of sexual preparation. Thus, the relatively low sperm output values do not reflect possible effects of varying energy intake on the rate of sperm production. However, sperm output and extra-gonadal reserves as measured by depletion trials at 4 yr. of age (Table 4) were not markedly affected by restricted energy intake. Future studies of this type should incorporate more demanding collection frequencies, with intensive preparation, in order to detect possible changes in semen characteristics and spermatogenesis (9). Using a partial exhaustion technique to provide a more demanding collection frequency, VanDemark *et al.* (21) recently reported that the number of sperm collected from two underfed 3-yr.-old bulls was 77% of that collected from the normally fed controls. Furthermore, reversal of the rations at 4 yr. of age did not correct deficiencies in the number of sperm collected from the previously underfed bulls.

It would have been interesting to continue observation of these bulls over a longer period of time, particularly in light of the effects of level of feeding on the fertility, onset of disease, and longevity of rats (2, 3, 15). From a practical standpoint, however, the development of weaknesses of the feet and legs and the lower level of sexual activity in the H group suggest that these factors would soon limit the useful life of these bulls.

Level of energy intake during early life apparently had little effect on the quality or quantity of semen produced later. Those bulls changed from L to H at 2 yr. of age did not show the slow sexual reaction time of those continually on H; sluggishness may have developed in LH bulls, had the high energy feeding been continued over a longer period of time. In fact, some indication of this was observed in the depletion trials. A program of heavy feeding during early life followed by restricted feeding later may have some merit. By moderate to

heavy early feeding, semen production is initiated at an early age, while restricted later feeding should prolong the useful life of the bull.

It is apparent that high energy intake is not desirable for bulls past the stage of rapid growth, while subnormal energy intake in early life is disadvantageous, in that it delays the onset of semen production. Early liberal feeding, alone or in combination with hormonal stimulation (6), may be economically feasible (4) when it is imperative that semen be obtained at the earliest possible age.

ACKNOWLEDGMENT

The authors wish to acknowledge the cooperation of personnel at the Western Pennsylvania Artificial Breeding Cooperative, Clarion, and the Southeastern Pennsylvania Artificial Breeding Cooperative, Lancaster, in conducting the fertility phase of this investigation.

REFERENCES

(1) Asdell, S. A., Bratton, R. W., Frank, A. H., Mixner, J. P., Pfau, K. O., Plastridge, W. N., and Yates, V. J. Breeding Difficulties in Dairy Cattle—Their Causes and Prevention. Cornell Univ. Agr. Expt. Sta., Bull. 924. 1957.

(2) Berg, B. N. Nutrition and Longevity in the Rat. I. Food Intake in Relation to Size, Health and Fertility. J. Nutrition, 71: 242. 1960.

(3) Berg, B. N., and Simms, H. S. Nutrition and Longevity in the Rat. II. Longevity and Onset of Disease with Different Levels of Food Intake. J. Nutrition, 71: 255. 1960.

(4) Bratton, R. W., Musgrave, S. D., Dunn, H. O., and Foote, R. H. Causes and Prevention of Reproductive Failures in Dairy Cattle. II. Influence of Underfeeding and Overfeeding from Birth to 80 Weeks of Age on Growth, Sexual Development, and Semen Production of Holstein Bulls. Cornell Univ. Agr. Expt. Sta., Bull. 940. 1959.

(5) Casida, L. E. Effect of Feed Level on Some Reproductive Phenomena in Cattle, Sheep, and Swine. Reproductive Physiology and Protein Nutrition, p. 35. Rutgers University Press. 1959.

(6) Davies, D. V., Mann, T., and Rowson, L. E. A. Effect of Nutrition on the Onset of Male Sex Hormone Activity and Sperm Formation in Monozygous Bull-Calves. Proc. Roy. Soc., 147: 332. 1957.

(7) Flipse, R. J., and Almquist, J. O. Unpublished data. Pennsylvania Agr. Expt. Sta. 1959.

(8) Flipse, R. J., Snyder, J. W., Thacker, D. L., and Almquist, J. O. Effect of Total Digestible Nutrient Intake on Growth and Reproductive Development of Dairy Bulls. Pennsylvania Agr. Expt. Sta., Prog. Rept. 104. 1953.

(9) Hale, E. B., and Almquist, J. O. Relation of Sexual Behavior to Germ Cell Output in Farm Animals. The Effect of Germ Cell Damage on Animal Reproduction. J. Dairy Sci., 43: Supplement, p. 145. 1960.

(10) James, J. P. The Effect of Level of Nutrition on the Sexual Capacity of Dairy Bulls. Proc. New Zealand Soc. Animal Production, 10: 84. 1950.

(11) Kordts, E., and Hildebrandt, H. H. Untersuchungen uber unterschiedliche Aufzuchtintensitaten an Zwillingsbullen unter besonderer Beruck-sichtigung ihres spateren Befruchtungsvermogens (Spermaqualitat). Kiel. Milchev. Forsch-Ber., 10: 481. 1958. [Abstract from Animal Breed. Abstrs., 28: 670.]

(12) Loosli, J. K., Huffman, C. F., Petersen, W. E., and Phillips, P. H. Recommended Nutrient Allowances for Domestic Animals. III. Recommended Nutrient Allowances for Dairy Cattle. Natl. Research Council Rept. 1950.

(13) Mann, T., and Rowson, L. E. A. Effect of Different Planes of Nutrition on the Composition of Bull Semen. 3rd Intern. Congr. Animal Production, 1: 21. 1956.

(14) MANN, T., AND WALTON, A. The Effect of Underfeeding on the Genital Functions of a Bull. J. Agr. Sci., 43: 343. 1953.

(15) MCCAY, C. M., CROWELL, M. F., AND MAYNARD, L. A. The Effect of Retarded Growth upon the Length of Life Span and upon Ultimate Body Size. J. Nutrition, 10: 63. 1935.

(16) MORRISON, F. B. Feeds and Feeding. 22nd ed. Morrison Publ. Co., Ithaca, N. Y. 1956.

(17) OLSON, H. H. Uniformity and Nutritional Studies with Monozygous Bulls. J. Dairy Sci., 35: 489. 1952. (Abstr.)

(18) REECE, R. P. Role of Nutrition in Mammary Physiology. Reproductive Physiology and Protein Nutrition, p. 23. Rutgers University Press. 1959.

(19) REID, J. T. Effect of Energy Intake upon Reproduction in Farm Animals. J. Dairy Sci., 43: Supplement, p. 103. 1960.

(20) VANDEMARK, N. L., AND MAUGER, R. E. Factors Affecting Replenishment of Sperm Numbers in Bulls Frequently Ejaculated. J. Animal Sci., 17: 1215. 1958. (Abstr.)

(21) VANDEMARK, N. L., FRITZ, G. R., EWING, L. L., AND MAUGER, R. E. Limiting Effects of Underfeeding During Growth on Sperm Production of Mature Bulls. J. Animal Sci., 19: 1338. 1960. (Abstr.)

(22) WINCHESTER, C. F., AND HOWE, P. E. Relative Effects of Continuous and Interrupted Growth of Beef Steers. USDA Tech. Bull. 1108. 1955.

ELECTRON MICROSCOPIC STUDY OF MATURE BOVINE SPERMATOZOA [1]

DONALD F. RAHLMANN

Department of Animal Husbandry, University of California, Davis

SUMMARY

Characteristics of the normal bovine spermatozoa as revealed by electron micrographs have been reported. Detail of the head area reveals that the acrosomic granule is an integral structure within the acrosomic cap of the mature spermatozoa. The acrosomic cap overlaps the porous-appearing posterior nuclear cap. The midpiece containing outer axial fibrils of at least two different dimensions appears to have a diameter at least as great as the thickness of the head. Possible contractile elements of the fibrils are closely associated with the basal granules in the neck. Elements of the tail area also have been described.

The electron microscope has revealed many of the nuclear and cytoplasmic changes occurring with the development of the spermatid. However, certain of the structural elements in the mature bull spermatozoa that should be present, if our present concept of spermatozoa development is correct, have not been fully demonstrated.

Baylor, Nalbandov, and Clark (2) in 1943, and Reed and Reed (6) in 1948 reported observations on whole unstained bull sperm. From 1947 through 1955, Bretschneider (4, 5) published a series of papers on the microstructure of bull spermatozoa. The shadow-casting technique was used extensively in his specimen preparation. Wu and McKenzie (7) employed this technique in association with chemical denudation to demonstrate organizational elements of the sperm cell in several mammalian species, including the bovine. Few investigators (3, 8) have reported on imbedded and sectioned mature spermatozoa of the bull.

EXPERIMENTAL PPROCEDURE

Semen samples were collected from a Jersey bull of proven fertility by means of an artificial vagina. Estimates of motility, concentration, and percentage of abnormal forms were determined for each sample. Percentage abnormal forms, as determined by the light microscope, never exceeded 5% (mostly tailless) in any sample. Fixation for 15 min. was carried out at room temperature (22 to 24° C.) in 1% osmic acid solution adjusted to a pH of 7.2 with veronal acetate buffer. Fixation was followed by three washings in veronal acetate buffer. The specimens were dehydrated in a graded series (20% through absolute) of ethanols at 15-min. intervals. Dehydration was followed by 1:1 absolute ethanol and a solution of five parts butylmethacrylate and one part methylmethacrylate with 2% Luperco CDB catalyst. Three changes were then made in the 5:1 butylmethacrylate and methylmethacrylate mixture which was

Received for publication January 3, 1961.

[1] This investigation was supported in part by United States Public Health Service Grant RG 5868.

slightly prepolymerized by oven heat at 70° C. for 10 min. The prepolymerization treatment tended to minimize damage to cell structure. In preliminary trials without prepolymerization, cell damage, particularly extreme ballooning or explosion artifact of the outer cytoplasmic membrane and head cap, was noted. A small portion of the prepared specimen was placed in No. 00 gelatin capsules. The capsules were filled with the methacrylate mixture, covered, and placed in an oven (60-70° C.) for 72 hr.

Three or four days after removal from the oven, the gelatin capsule was removed by soaking in water and a double-truncated pyramid surrounding the specimen area was cut with a sharp, single-edged razor.

Thin sections were cut by a glass knife with a Porter-Blum ultramicrotome and mounted on formvar-coated 200-mesh copper grids. Some of the grid-mounted specimens were subsequently stained by flotation for varying periods of time on solutions of 4% potassium permanganate, 1% lanthanum sulfate, 1% phosphotungistic acid, or 4% potassium dichromate neutralized with potassium hydroxide. Examination was made, without removing the imbedding plastic, in an RCA-EMU3D electron microscope. Micrographs were taken at magnifications ranging from 2,500 to 15,500. Higher magnifications were obtained by photographic enlargement.

RESULTS AND DISCUSSION

Osmic acid alone or osmic acid in combination with potassium permanganate seemed to be superior to other stains used in this study. A saggital section of a single spermatozoa extending from the head through the midpiece and the anterior portion of the tail is shown in Figure 1. An outer cytoplasmic membrane completely surrounds the sperm. In part, particularly in the head area, this membrane appears to be double-walled. The anterior head cap formed from the acrosomic vesicle fits closely to the nuclear membrane and overlaps the posterior nuclear cap. Apically, the head cap thickens and presents a concave profile on one aspect (probably ventral) a short distance posteriorally. An additional structure, presumably the acrosome, can be distinguished within the head cap in Figure 2.

The postnuclear cap, closely surrounding the posterior position of the nucleus, appears as a series of short, dark

FIG. 1. Saggital section of the bovine spermatozoa. Detail of head, midpiece, and anterior portion of the tail described in text and in other micrographs. 1% OsO_4. × 7,250.

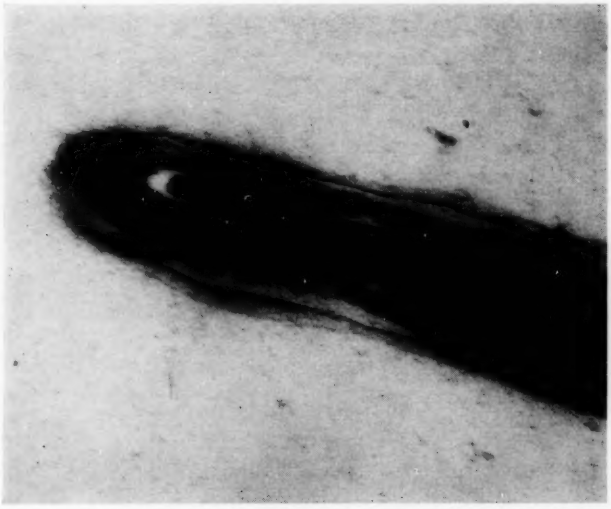


FIG. 2. Apex of head with acrosome (in front of the clear area) within anterior head cap. 1% OsO_4 plus 4% $KMnO_4$. × 62,000.

lines periodically interrupted by clear spaces. Thus, it appears to be more porous than other membranes associated with the head area. This feature may account for its permeability to certain dyes in the dead spermatozoa.

The dense chromatin material of the nucleus is thickest near the junction of the midpiece and gradually tapers anteriorally.

The basal granules (Figure 3), apparently three in number, are partially inserted within the head. The medial granule penetrates to the greater extent.

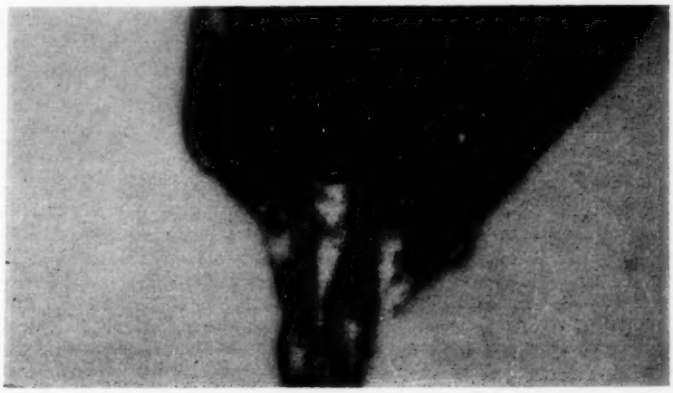


FIG. 3. Junction of the head and midpiece. Note transverse banding on fibrils attached to basal granules which are inserted within the head (center of micrograph). 1% OsO_4. × 30,000.

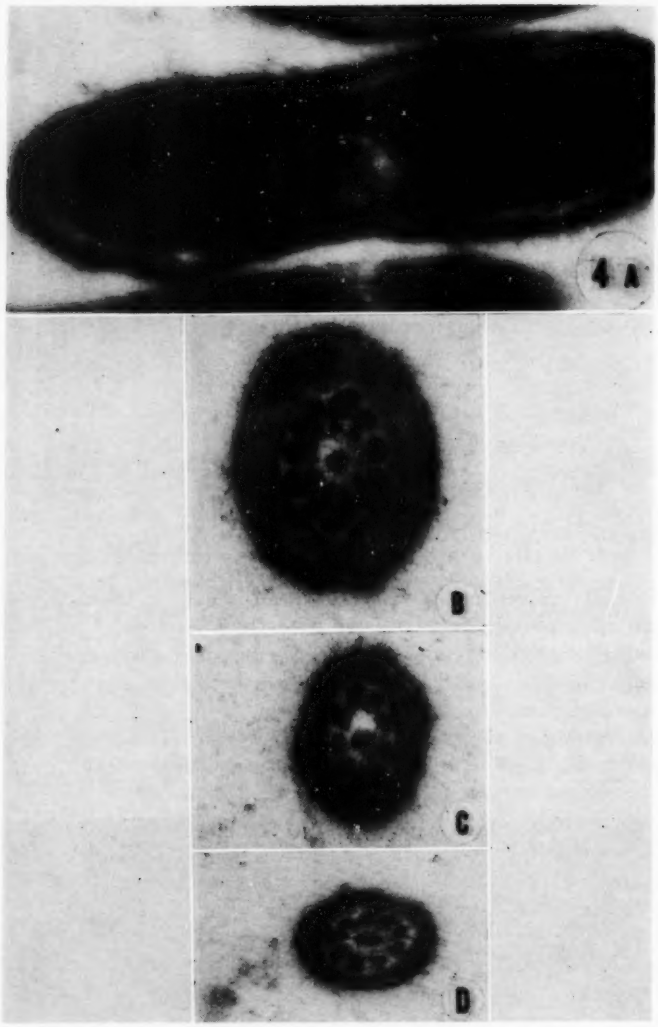


FIG. 4. Cross sections of spermatozoa. a) Base of head with insertion of medial basal granule. b) Midpiece. Mitochondrial sheath surrounding two concentric circles of nine outer axial fibrils, the larger located peripherally, and a set of two fibers in the center of the section. c) Tail section with longitudinal rib structures in the same plane as central fibrils. Several fine granular lines proceed radially from the central pair of fibrils. d) Tail section posterior to level at c). Small projections can be seen on the periphery of the outer axial fibers. 1% OsO_4 plus 4% $KMnO_4$. × 62,000.

Periodical transverse banding appears on the fibrils attached to the granules. The bands appear similar to the contractile elements described in striated muscle. The banding was detected only in the fibrils within the proximal portion of the midpiece. Wu and McKenzie (7) have reported the presence of these structures in sperm which have been chemically denudated. Bretschneider (4) noted cross striations and considered them to be artificial fragmentation of band structures in membranes of the neck region. In the present study, the periodicity of these striations, and the frequency of their appearance in a number of different planes of sectioning, plus a consideration of the nature of the region immediately surrounding the axial fibrils, tend to indicate that these structures are an integral part of the sperm and not of artifactual origin.

The mitochondrial sheath of the midpiece is coiled with a pitch of 25° approximately 75 times around the axial fibrils. Within the midpiece and tail, two fibrils surrounded by two concentric circles of nine fibrils are found (Figure 4). At least three, possibly four, of the fibrils are larger than the others in the

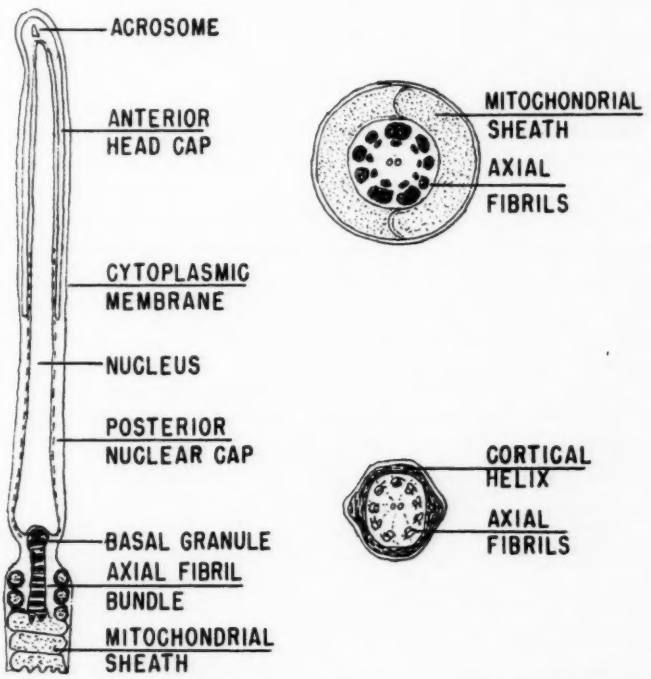


FIG. 5. Schematic diagram of the mature bull spermatozoa as conceived from micrographs observed during the course of this study. a) Saggital section of the head, neck, and anterior portion of the midpiece. Compare with Figures 1 and 2. b) Cross section of the midpiece. Compare with Figure 4a. c) Cross section of the tail and longitudinal ribs, apparently a thickening of the cortical helix, in the same plane as the central pair of fibrils. Compare with Figures 4 c and 4 d.

midpiece area. The apparent difference in cross-sectional area seems to diminish as the fibrils proceed posteriorally along the tail. The diameter of the midpiece appears to be greater than the thickness of the head. If the head and midpiece can be considered nondifferentially enlarged or shrunken by the preparative processes employed, the head measures between 650 and 700 mμ in thickness. Head thickness in bovine sperm has been difficult to determine by either light or phase contrast microscopy.

The cortical helix proceeds from the midpiece posteriorally on the tail, appearing as a regular series of dark bands in Figure 1. Longitudinal ribs, apparently a thickening of the cortical helix (Figure 4 c, d) in the same plane as the central pair of fibrils, are found in the main piece of the tail.

Short projections of the peripheral axial fibrils appear in cross sections of the tail area (Figure 4 c, d). They may possibly form connecting links between the fibrils or the fibrils and the cortical helix. In addition, radial spokes of a granular character can be seen proceeding from the central pair of fibrils. The character of these subunits appears to be similar to that described by Afzelius (1) for sea-urchin sperm.

Schematic line drawings of certain structural aspects of the bull spermatozoa are presented in Figure 5.

ACKNOWLEDGMENT

The author thanks J. Pangborn of the Davis campus, University of California Electron Microscopy Laboratory, for his aid and advice in the preparation of the photomicrographs.

REFERENCES

(1) AFZELIUS, B. Electron Microscopy of the Sperm Tail. Results Obtained with a New Fixative. J. Biophys. Biochem. Cytol., 5: 269. 1959.

(2) BAYLOR, M. R. B., NALBANDOV, A., AND CLARK, G. A. Electron Microscope Study of Sperm. Proc. Soc. Exptl. Biol. Med., 54: 229. 1943.

(3) BRADFIELD, J. R. G. The Structure of Mammalian Spermatozoa. Proc. Intern. Conf. Electron Microscopy. p. 599. London, 1954.

(4) BRETSCHNEIDER, L. H. Die morphogenese and Pathogenese der Spermien vom Stier. II. Koninkl. Ned. Akad. Wetenschap., Proc. Ser. C., 58: 509. 1955.

(5) BRETSCHNEIDER, L. H., AND VAN ITERSON, W. An Electron Microscopical Study of Bull Sperm. I. Koninkl. Ned. Akad. Wetenschap., 50: 88. 1947.

(6) REED, C. I., AND REED, B. P. Comparative Study of Human and Bovine Sperm by Electron Microscopy. Anat. Record, 100: 1. 1948.

(7) WU, S. H., AND MCKENZIE, F. Microstructure of Spermatozoa After Denudation as Revealed by the Electron Microscope. J. Animal Sci., 14: 1151. 1955.

(8) YASUZUMI, G. Spermatogenesis in the Animal as Revealed by Electron Microscopy. III. Formation and Submicroscopic Structure of the Tail Flagellum of Fish, Bird, and Mammalia. Regional Conf. Electron Microscopy in Asia and Oceania, 1st Proc. 1956

PART LACTATIONS. III. PROGENY TESTING WITH PART LACTATION RECORDS

S. R. SEARLE

New Zealand Dairy Board, Wellington

SUMMARY

Analyses of monthly records of artificially-bred cows indicate that under New Zealand conditions records accumulated up to a specified test day early in lactation are suitable for progeny testing. Heritability slightly exceeds that for lactation yield and the genetic correlation between the part record and lactation yield exceeds 0.90. As a result, the rate of progress in improving additive genetic merit for lactation yield when selecting on part records differs very little from when selecting on lactation yield. Approximately 40, 60, and 80 daughters in sire proofs using part records give the same information about lactation yield as a proof using lactation yield on 30, 40, and 50 daughters, respectively. Proofs evaluated on both kinds of records for the same group of sires were highly correlated and the part lactation proofs were just as repeatable as those based on lactation yields.

Earlier papers of this series, (6) and (7), have reported age-correction factors and estimates of genetic and phenotypic parameters for monthly milk fat yields. The use of such records for progeny testing is now considered. First, heritability estimates are reported for records accumulated up to a specified test day, together with the genetic correlation of these records with lactation yield. These enable the rate of progress in improving genetic merit for lactation yield that could be achieved when selecting on part records to be compared with that which could be achieved when selecting on lactation yield itself. Secondly, methods are developed and used for finding the number of daughters required in a sire proof using part records, for the proof to contain the same amount of information on the sire's genetic merit for lactation yield as does a proof based on a given number of lactation yield records. Finally, comparisons are made of actual progeny tests calculated from part lactation records with those calculated from the corresponding complete lactation yields. This comparison indicates to what extent the two methods of progeny testing differ.

Monthly records could be used for progeny testing in many ways—a single month, the first two, three, or more months, alternate months, and numerous other combinations. This paper is a study of records accumulated up to a fixed date. A fourth (and final) paper in this series will deal with a variety of combinations of lactation months.

DATA AND CORRECTION FACTORS

The basic data are described in (6), the study being confined to records from cows first tested in August (60%), September (30%), or October (10%). Cows first tested in these months represent 90% of the cows tested in any particular year, as indicated in (3).

The earlier proving of sires would be one advantage of progeny testing with

Received for publication December 18, 1960.

part lactation records which could be achieved by using monthly yields accumulated up to a specified test day. Three possible alternatives suitable for this purpose in New Zealand are to accumulate yield for all cows up to the test day in either November, December, or January. For cows first tested in August (the predominant group) this would mean part lactations of 4, 5, or 6 mo., respectively; those first tested in September would have 1 mo. less and October-starting cows 1 mo. less still. These three possibilities were investigated in this study, using correction factors to take account of lactation-length differences simultaneously with age, in each case. These correction factors and the mean production averages from which they were derived are shown in Table 1.

The means in Table 1 are of milk fat yield up to and including the November, December, and January test, for cows first tested in August, September, and October. They were accumulated from the test-day milk fat yields multiplied by 30 (to bring them to a monthly equivalent), a proportional amount being used to estimate yield in the first month for the period from four days after calving to 15 days after the first test day. Two years' data are included in Table 1; 1956-7 production was lower than that of 1957-8, but the resulting correction factors showed only small differences between years. The first entry in the Table is 140; this is the mean yield up to and including the November test of 2-yr.-olds that started on test in August. The entries decrease along each row, as would be expected, the later-starting cows having fewer months on test. Likewise, the figures for December and January exceed those for November, because more months are included in the part lactation.

The correction factors correct all records to the level of mature cows (5-9-yr.-olds) first tested in August; for example, the part records up to November are corrected up to the 180-lb. average of the August-starting mature cows. Thus, the multiplicative factor for the 3-yr.-olds that started in September is $180/132 = 1.36$. These factors are shown in the right-hand section of Table 1. Those for the later-starting cows correct for number of months on test also, and

TABLE 1

Mean production (pounds milk fat) in early lactation; and multiplicative factors to correct part lactations for age and number of months on test up to the level of 5-9-yr.-olds first tested in August

Last month of part lactation	Age (yr.)	Mean production First month tested			Correction factor First month tested		
		Aug.	Sept.	Oct.	Aug.	Sept.	Oct.
November	2	140	113	76	1.29	1.59	2.37
	3	159	132	95	1.13	1.36	1.89
	4	174	142	104	1.03	1.27	1.73
	5–9	180	147	105	1.00	1.22	1.71
December	2	176	151	117	1.27	1.48	1.91
	3	198	174	141	1.13	1.28	1.58
	4	215	187	154	1.04	1.19	1.45
	5–9	223	194	156	1.00	1.15	1.43
January	2	208	186	152	1.26	1.41	1.72
	3	232	213	183	1.13	1.23	1.43
	4	252	228	199	1.04	1.15	1.32
	5–9	262	238	202	1.00	1.10	1.30

the factor for August-starting mature cows is, of course, 1.00. The records were corrected for the analysis studies, using these multiplicative factors converted to herd-level factors by the method explained in (5).

GENETIC PARAMETERS

The records of all daughter-dam pairs in the 1956-1957 data were accumulated up to November, December, and January and corrected in accordance with Table 1. Estimates of within-herd heritability (in the narrow sense) of these part records and of genetic correlations with lactation yield are given in Table 2. The heritability values all exceed the 0.19 estimate obtained from the same cows for lactation yield [see (7)] and the genetic correlations are all close to 1.00.

The right-hand column of Table 2 shows the relative rate of progress in improving additive genetic merit for lactation milk fat yield through mass selection of cows on part records relative to selection on lactation yield. These values have been calculated from the expression $r\sqrt{h/0.19}$, where h is the heritability of the part record and r is its genetic correlation with lactation yield, 0.19 being the heritability of lactation yield. This expression was also used by Madden *et al.* (2), whose results are very similar to those reported here. The conclusion is that selection of cows on part records will achieve progress in improving genetic merit for lactation yield almost as quickly as will selection on lactation yield. The rate of progress using records up to 6 mo. in length (ceasing in January) may even lead to slightly faster progress.

EQUIVALENT PROOFS

Two kinds of records available for progeny testing a sire can be compared by considering the relative amount of information about lactation yield that is in the proofs based on a given number of each kind of record. This is measured in each case by the correlation between the sire's estimated additive genetic merit for lactation yield and his actual additive genetic merit. The expression for this when using the customary selection index procedure with single records of n daughters is

$$r\sqrt{\frac{nh}{4+h(n-1)}}$$

TABLE 2
Genetic parameters for part lactations

Part lactation (milk fat yield)	Heritability [a]	Genetic correlation with lactation yield	Relative rate of progress in improving additive genetic merit for lactation yield when selecting on part yield
			(%)
Ceasing			
November	.21	.92	97
December	.21	.93	98
January	.23	.93	102

[a] Estimate for lactation yield is 0.19, taken from (7).

where h is the heritability of the trait observed in the daughters and r is the genetic correlation of this trait with that for which the sire's genetic merit is being estimated. This is the situation when indexing sires for lactation yield on part lactation records of their daughters: h is the heritability of the part record and r is the genetic correlation of the part record with lactation yield. The more usual form of this correlation as found, for example, in Henderson (1), is when the trait observed in the daughters is identical to that being estimated in the sire and r is then 1.00. This is the situation when indexing sires for lactation yield on the lactation yields of their progeny. Using 0.19 for the heritability of lactation yield, the correlation between the sire's additive genetic value and its prediction, based on the single lactation yields of N daughters, is then

$$\sqrt{\frac{0.19N}{4+0.19(N-1)}}.$$

These correlations measure the accuracy of two different estimates of the same thing in the sire. The sire proofs will, therefore, be of equal accuracy when the correlations are equal, i.e.,

$$r\sqrt{\frac{nh}{4+h(n-1)}} = \sqrt{\frac{0.19N}{4+0.19(N-1)}}.$$

Thus, a means is provided of finding the number of daughters n, needed to prove a sire on part lactation records, such that the proof will contain the same amount of information about the sire's additive genetic value for lactation yield as a proof based on the lactation yields of N daughters. The equation reduces to

$$n = \frac{19N(4-h)}{h[381r^2 - 19N(1-r^2)]}.$$

This is the method referred to by VanVleck (9), who has also developed an alternative, but essentially equivalent, procedure for solving the same problem.

Values of n (the number of part lactation records) corresponding to N (the number of lactation yield records) equal to 10, 20, 30, 40, 50, 75, and 100, are given in Table 3 for the three kinds of part lactation records used in this study, based on the heritabilities and genetic correlations shown in Table 2.

TABLE 3

Number of daughters required in a sire proof using part records to be equivalent to a proof using lactation yields

No. of daughters using lactation yields	No. of daughters using part lactation records ceasing in		
	November	December	January
10	12	12	11
20	26	25	23
30	44	41	38
40	67	61	55
50	98	85	78
75	249	188	171
100	1,119	471	428

This shows, for example, that a proof using 12 daughters with records accumulated through November is equivalent to a proof based on ten daughters with lactation yield; that 98 daughters with similar part lactation records are equivalent to 50 daughters with lactation yields, and so on.

The number of daughters required for part records exceeds the number required for lactation yields in all cases, and the increases in the number of daughters required get larger as the number with lactation yields increases; e.g., 12 part records (ceasing November) equal ten lactation yields, but 1,119 equal 100 lactation yields. This increase arises from the nature of the equation relating n to N. The denominator of n decreases with increasing N until it becames negative for values of N greater than $381r^2/19(1-r^2)$. This is clearly impossible because the numerator is always positive and n can not be negative. Hence, $381r^2/19(1-r^2)$ is an upper limit on N. Thus, there is a limited range of numbers of daughters in a proof for lactation yield using lactation yield, for which a proof using part records on more daughters can be as efficient; and this range depends on the genetic correlation of the part record with lactation yield. As N increases and approaches its upper limit, n increases very rapidly, so giving rise to the large numbers found in the last row of Table 3.

PROGENY TESTS

The data included the 1956-1957 records of 2,901 daughters by 51 sires used in artificial breeding and the 1957-1958 records of 4,195 daughters by 62 sires. Progeny tests were calculated for the sires each year on the basis of comparing each daughter record with a contemporary herd average that excluded the daughter record. The sire index was calculated as

$$\frac{n}{n+15}\,(D-0.90H-0.10R),$$

where n is the number of daughter records in the proof, D is the mean daughter production, H is the mean contemporary herd average, and R the mean regional average appropriate to the daughter average. The regional averages used in R are the averages of the Herd Improvement Associations responsible for all herd-testing work in New Zealand and the factor $n/(n+15)$ is the adjustment for daughter numbers, as developed in (4).

The 0.90 factor is the percentage of the difference in herd production levels that is reflected in the records of paternal half-sibs in different herds. This is the figure reported by Spalding *et al.* (8), estimated as the within-sire regression of daughter record on contemporary herd average. This was estimated for the part lactation records considered here and the results are shown in Table 4. The estimates for lactation yield which was corrected for age only as in (5), are 0.94 and 0.90 in the 2 yr., approximating closely to the 0.90 of Spalding *et al.* (8). The estimates for the part lactations showed greater differences between the years, decreasing in 1956-1957 and appearing to increase in 1957-1958 as the part lactation increases in length. The reason for this difference is not apparent and as an average value for all records (possibly a little low) the 0.90 figure was used.

TABLE 4

Intra-sire regression estimates of daughter record on contemporary herd average

Type of record	Regression estimates	
	1956–1957 (2,901 daughters, 51 sires)	1957–1958 (4,195 daughters, 62 sires)
Part lactations		
Ceasing		
November	.99	.85
December	.97	.92
January	.95	.91
Lactation yield	.94	.90

The progeny tests for the sires were evaluated in four ways, using the lactation yields and the three sets of part records. Those based on the part records were correlated each year with those calculated from lactation yield and the results are shown in Table 5 for sires having six or more daughters. The correlations are not significantly different from 1.00 in all cases and the differences between the years are not significant, although the estimates from 1957-1958 are all larger than those for 1956-1957. These results indicate that proofs based on part lactations rank the bulls no differently from proofs based on whole lactations. This is not necessarily an indication that part lactation proofs are better than lactation yield proofs, but that estimates of a bull's genetic value obtained from part lactations are similar to those obtained from whole lactations. The rank coefficients were also estimated, the results being very close to the product-moment values shown in Table 5.

Several of the bulls, 36 in fact, had daughters in both years and correlations between their proofs in the 2 yr. were also estimated. The results are shown in Table 5. No account was taken of the fact that many daughters having a record in 1956-1957 also had a record in 1957-1958. These correlations are significantly different from zero and, more particularly, those for the part lactation proofs do not differ significantly from that for the lactation yield proofs. This suggests that progeny tests based on part lactation are just as repeatable as those based on lactation yield. The conclusion is reached, therefore, that part lactations as calculated in this study give progeny test results

TABLE 5

Correlations between progeny tests

Last month of part lactation	Correlation of part lactation progeny test with lactation yield progeny test		Correlation between progeny tests in 2 yr. for 36 bulls [a] (s.e. = 0.17)
	1956–1957: 42 sires (s.e. = 0.16)	1957–1958: 62 sires (s.e. = 0.13)	
November	.76	.85	.69
December	.83	.90	.79
January	.88	.93	.81

[a] Value for lactation yield ratings: 0.82.

for bulls used in artificial breeding very similar to lactation yields and that such progeny tests are as equally repeatable as those based on lactation yields. This would seem to recommend part lactations as suitable for progeny testing.

ACKNOWLEDGMENT

The statistical analyses reported in this paper were supported by funds from a research grant entitled Estimates of Genetic Parameters from the National Science Foundation of the U.S.A. Sincere thanks are given to Dr. C. R. Henderson of Cornell University for this support and for the generous use of facilities which made this study possible.

REFERENCES

(1) Henderson, C. R. Mimeographed 120 Class Notes, Cornell University, Ithaca, N. Y. 1956.
(2) Madden, D. E., Lush, J. L., and McGilliard, L. D. Relations Between Parts of Lactation and Producing Ability of Holstein Cows. J. Dairy Sci., 38: 1264. 1955.
(3) New Zealand Dairy Board. Annual Report. 1951.
(4) Searle, S. R. Use of the Selection Index Method to Account for the Number of Daughters and Records in Progeny-Testing a Dairy Bull. J. Dairy Sci., 42: 1666. 1959.
(5) Searle, S. R. Simplified Herd-Level Age-Correction Factors. J. Dairy Sci., 43: 821. 1960.
(6) Searle, S. R. Part Lactations. I. Age-Correction Factors for Monthly Milk Fat Yields. J. Dairy Sci., 44: 104. 1961.
(7) Searle, S. R. Part Lactations. II. Genetic and Phenotypic Studies of Monthly Milk Fat Yield. J. Dairy Sci., 44: 282. 1961.
(8) Spalding, R. W., Carter, W., and Henderson, C. R. Evaluation of A.B. Production-Tested Sires. Cornell Ext. Bull. 999, Cornell University, Ithaca, N.Y., 1957.
(9) VanVleck, L. D. The Value of Part Lactation Records in Selection. Ph.D. thesis, Cornell University, Ithaca, N. Y. 1960.

ADRENALECTOMY OF THE CALF AND ITS EFFECTS ON VARIOUS BLOOD CONSTITUENTS [1]

V. L. ESTERGREEN, JR.[2] AND N. L. VAN DEMARK
Department of Dairy Science, University of Illinois, Urbana

SUMMARY

Surgical adrenalectomy was successfully performed in calves without suturing the wall of the vena cava, by using a special forceps to facilitate removal of the gland. Maintenance of adrenalectomized calves was possible under controlled conditions, with the intramuscular injection of 25 mg. cortisone acetate or 5 mg. prednisone plus 5 mg. desoxycorticosterone acetate per 100 lb. body weight, daily or every other day. Calves were also maintained in an apparently normal state without steroid therapy by the free-choice feeding of salt, which was consumed at a rate about four times that of the normal calf.

The outward symptoms of adrenal deficiency, manifested during the period when no therapy was given, began with lethargy and weakness in the legs. This was followed by anorexia, extreme apathy, a completely prostrate position, and extreme depression and arrhythmia of the heart beat shortly before death ensued. Loose, foul-smelling feces and a rapid, labored respiration were sometimes observed. The calves survived about six to seven days following withdrawal of maintenance therapy.

In three adrenal-deficient calves there was a decrease in the serum Na:K ratio, a decrease in serum chlorides, and an increase in serum potassium, all of which were highly significant (P less than 0.01). In two of the three animals there was a reduction in serum sodium, total neutrophils, and mature neutrophils, and an increase in RBC and eosinophils. The influence of adrenalectomy on serum calcium, blood hemoglobin, hematocrit, and neutrophil levels varied considerably between calves. No significant differences were found in WBC, lymphocyte, and monocyte levels following adrenalectomy.

One calf was found to have an adrenal remnant which prevented it from succumbing completely to the effects of adrenalectomy.

One approach to the study of adrenal gland function in an animal is to investigate the changes which occur in the animal after removal of the gland. Then, by substitution therapy with adrenal steroids, the levels of these hormones needed for normal maintenance of the animal can be determined.

Prior to the present study, only one instance of successful surgical adrenalectomy of a bovine had been reported in the literature. In 1957, Holcombe (10) reported the adrenalectomy of a bull in a study of the urinary glucocorticoid excretion. Mansson and Obel (15) later reported on the surgical techniques employed. In 1959, Estergreen and VanDemark (6) made a preliminary report on blood changes in an adrenalectomized calf. Recently, Cupps *et al.* (5) reported the successful adrenalectomy of two bull calves and two mature bulls.

Adrenalectomy in the bovine presents some technical problems because of the firm attachment of both adrenals to the wall of the posterior vena cava (15,

Received for publication January 13, 1961.

[1] The data presented in this paper were taken from a thesis submitted by the senior author to the Graduate College of the University of Illinois in partial fulfillment of the requirements for the Ph.D. degree.

[2] Present address, Department of Biochemistry, College of Medicine, University of Utah, Salt Lake City, Utah.

23). These glands have no adrenal vein or a very short one, so special techniques must be devised for their removal without excessive hemorrhage. Cowie and associates (3, 4), working with goats, described special clamps which could be applied between the gland and the wall of the vena cava which would aid in removal of the gland and in suturing the defect left in the posterior vena cava. Mansson and Obel (15) describe a similar use of long, curved artery forceps for aid in removal of the adrenal gland from a bull.

The present study was undertaken in an attempt to a) adapt surgical procedures to removal of the adrenal glands of the calf, b) investigate the levels of replacement hormones needed to maintain the adrenalectomized calf, c) study the possibilities of maintaining the adrenalectomized calf on salt alone, and d) examine the effects of adrenal deficiency on several blood components of the calf.

SURGICAL PROCEDURE

Young calves were selected for this study because the depth of field in which to work was less, there was less interference from the rumen, and interfering perirenal fat deposits were absent as compared with older animals (15). The calves were fasted and kept from water for 24 hr. before surgery. Intravenous sodium pentobarbital (Nembutal), accompanied by ether as needed during the operation, was the anesthetic used on the first calves. Later, a commercial combination of sodium pentobarbital, chloral hydrate, and magnesium sulfate (Equitol) given intravenously proved more effective. A stimulant, amphetamine sulfate (Amphetasul), was given immediately post-operatively to hasten recovery from the anesthetic.

The site of operation was prepared by close clipping with small animal clippers. Just before draping, the area was washed with soap and water, dried, and disinfected with iodine and alcohol. Bilateral adrenalectomy was performed one side at a time, allowing 2 wk. or more for recovery between operations. The left adrenalectomy was done first, to avoid the interference of the developing rumen.

The approach to the left gland was made through an 8- to 10-in. oblique incision, starting close to the first lumbar transverse process and running parallel to the last rib. Since the right adrenal was more anterior, approach was made to it through an incision between the last two ribs on the right side, starting as high as possible and keeping the incision close to the anterior adge of the last rib to clear the intercostal blood vessels.

The wound was spread with an adjustable abdominal retractor and on the left side the viscera were packed off with wet toweling and held back with additional retractors. On the right side, a lobe of the liver directly over the gland was carefully dissected free and retracted, exposing the gland directly under the incision.

The gland was freed from surrounding tissue by blunt dissection and all small blood vessels were doubly ligated and cut between ligations. The median border of the gland was carefully dissected free of the posterior vena cava except for the anterior one-third around the adrenal vein. The gland was lifted

and a Herrick kidney forceps clamped between the gland and the vena cava. The gland was then freed by running a scalpel along the outer edge of the kidney forceps. No suturing was done on the vena cava and the clamp was removed after about 5 min. without hemorrhage.

Normal suture and clamping techniques were used for closing the wound. A gauze and collodion dressing was placed over the wound to keep out dirt and insects. Three hundred thousand I.U. penicillin and 250 mg. dihydrostreptomycin were administered intramuscularly immediately post-operatively and daily for three to five days following. After removal of the second adrenal, steroid substitution therapy was begun. Of four calves bilaterally adrenalectomized, only one (H-3) was lost, presumably from shock, 18 hr. after surgery.

TREATMENTS AND METHODS OF ANALYSIS

Following surgery the first treatment consisted of maintenance of the animal by substitution steroid therapy. The second treatment consisted of maintenance of the animal by supplemental free-choice salt feeding without steroid therapy. The third treatment was the no-therapy period, in which the animal was allowed to go into the extreme deficiency state.

During all the periods of treatment the calves were kept in pens in a heated room and subjected to no more stress than was necessary for blood sampling. Two of the calves were fed milk twice daily for the entire period. The third calf was weaned before removal of the second adrenal. A high-quality calf starter grain mix, good-quality hay, and water were kept before the calves at all times.

Blood samples were taken from the jugular vein from five to 18 times during each treatment period on each calf. Results of the analysis on blood components were compiled for each treatment period and only the means are presented in this paper. The serum sodium, potassium, and calcium were determined by means of a Coleman flame photometer, serum chloride by the method of Whitehorn (24), hemoglobin by a visual comparison test using the Haden-Hausser technique (2), and the hematocrit as the percentage of packed cells following centrifugation at 2,500 r.p.m. for 30 min. A differential count of the white blood cells was made after staining with Wright's stain (2). Total red and white blood cells were counted visually on a hemacytometer. Statistical analysis was carried out as described by Ostle (18).

RESULTS AND DISCUSSION

In all the calves used in this investigation (ranging in age from 3 to 15 wk. at the time of surgery) both adrenals were found to be securely encapsulated to the posterior vena cava with a tough fibrous connective tissue. The right adrenal was consistently found to be 1 or 2 in. more anterior than the left and each was found close to the anterior pole of the kidney. Both adrenals were found slightly anterior to the location described by Smollich (23) and in somewhat differing positions from that described by Sisson and Grossman (22). Since these authors describe the position in adult animals, these differences may have been due to age.

The left gland had a very short adrenal vein (1-2 mm.) between the gland and the posterior vena cava. The right gland had no adrenal vein but communicated directly from the lumen of the gland into the posterior vena cava via a hole in the wall of the blood vessel.

Following early attempts in this experiment at ligating the adrenal vein or suturing the vena caval wall after removal of the gland, a Herrick kidney forceps was used, as described earlier. This forceps, similar to the clamp described and used by Cowie and Tindal (4) on goats, has two bends in the blade, making it easy to insert under a deep gland. On the inside, and running the length of each blade, are longitudinal ridges which crimp the tissue held between the blades. After clamping the vena cava for 5 min. with this forceps, it was unnecessary to suture the slit in the caval wall. No caval hemorrhaging was noted in the five operations where this clamp was used.

Steroid therapy. Daily intramuscular administration of approximately 25 mg. cortisone acetate (C.A., Schering Cortogen), or an equivalent amount of prednisone (Schering Meticorten), plus 5 mg. desoxycorticosterone acetate (DOCA, Schering Cortate) per 100 lb. body weight, maintained these adrenalectomized calves in an apparently normal condition. Cortisone acetate alone, at the levels given, did not prevent the onset of deficiency symptoms four days after adrenalectomy in Calf H-1. It was necessary to include injections of DOCA to maintain normal appearance in this calf and, consequently, both steroids were used for maintenance of the other calves in the experiment. This level of steroid substitution therapy is similar to the reports for maintenance of other adrenalectomized ruminants (4, 8, 10).

Although this level of steroid substitution therapy supported these calves under the controlled conditions, it was not adequate for coping with a severe stress. In one instance, the insertion of a venous cannula caused an animal to collapse. A full day was required for recovery following immediate steroid therapy. No information is available on the level of substitution therapy needed to support an animal under normal barn conditions or when penned with other animals.

Salt therapy. Free-choice salt feeding successfully maintained these adrenalectomized calves without substitution steroid therapy for 2 wk. There was every indication that Calves H-1 and G-5 could have been maintained for a much longer period, since no sign of deficiency appeared until at least four days following removal of the supplemental salt. The other calf, H-2, stopped eating salt after five or six days, but until that time no outward symptoms of deficiency were apparent. The high serum levels of Na and Cl exhibited during this salt-feeding period indicate the calf was successfully overcoming the excessive salt loss due to steroid deficiency. The salt consumption of one calf (G-5) was recorded for a ten-day period, and grain consumption determined for five days. During this time an average of 36 g. salt, 0.5 lb. grain, 9 lb. milk, and some hay were consumed daily. The total sodium chloride intake during this period was calculated to be 47 g. daily, exclusive of what was provided in the hay. This was more than four times the calculated salt consumed during the steroid therapy period.

These findings indicate that supplemental salt feeding alone can alleviate the outward manifestations of adrenal deficiency and maintain life in the adrenalectomized calf, as has been found in the adrenal-deficient rat, dog, and man (13, 14, 19). This concurs with the findings of Holcombe (10), who maintained an adrenalectomized bull four days by giving free access to rock salt.

Adrenal insufficiency. The outward manifestations of adrenal insufficiency began with a lethargy and a weakness in the legs, followed by a loss of appetite and extreme apathy. If no therapy was initiated the calves became almost completely comatose shortly before death. Other symptoms were loose, foul-smelling feces and a rapid, labored respiration before death. Significant was the sudden, extreme drop in heart rate and an irregularity of the heart beat. In one calf the heart rate dropped in a few hours from a normal of about 100-110 beats per minute to about 50-55 and was very irregular at the time the calf was prostrate. At this stage, intramuscular steroid therapy was ineffective in restoring the animal to normal. Intravenous infusion of 300-500 ml. of 25% glucose made up in physiological saline was effective in bringing the animal to his feet and alleviating the deficiency symptoms.

Calves H-1 and H-2 exhibited symptoms of adrenal deficiency after four days and survived seven and six days, respectively, following withdrawal of maintenance therapy. Death was due to extreme adrenal deficiency in both cases and post-mortem examination revealed no remnant of adrenal tissue at the sites of operation in either animal.

Calf G-5 exhibited signs of deficiency on the fifth day following cessation of maintenance therapy and was in an extremely deficient state by the seventh day. Some recovery occurred on the eighth day and on the tenth day following cessation of maintenance therapy the calf was sacrificed. Histological investigations revealed a small nodule of adrenal tissue embedded in the wall of the vena cava at the site of the right adrenalectomy.

Changes in blood components. The mean changes in some of the blood constituents for each treatment period are given in Table 1. The effects of adrenal deficiency on the serum electrolytes in these calves, a decrease in Serum Na and Cl, and an increase in Serum K, appear to follow closely the effects recorded for rats, dogs, goats, and sheep (3, 4, 8, 13, 14).

Although Serum Na and K levels were near normal during the nondeficient periods in this experiment, there was a significant decrease in the Serum Na : K ratio and in Serum Cl level, and a significant rise in Serum K in all three calves during adrenal deficiency (see Figure 1). In two of the three calves this was accompanied by a significant decrease in Serum Na. In the third calf, H-2, the Serum Na did not fall significantly, but there was a tremendous increase in Serum K (to 71.8 mg/100 ml.). Thus, the Serum Na : K ratios were similar for all three calves.

Probably the most constant finding in all the calves was the fall in Na : K ratio during the adrenal-deficient period. The mean Na : K ratio range of 13.7-16.9 for these calves during the periods before bilateral adrenalectomy and during the steroid and salt therapy periods was only slightly lower than

TABLE 1

Effect of adrenalectomy and subsequent steroid and salt therapy on some blood components of three calves
(Treatment means)

Blood component	Calf No.	Before adrenal-ectomy	Unilateral adrenal-ectomy	Steroid therapy	Salt therapy	No therapy
Serum	H1	.....	.....	314	314	281**
Na	H2	.....	354	345	371**	348
(mg/100 ml.)	G5	363	328**	325*	316**	278**
Serum	H1	.....	.....	22	23	29**
K	H2	.....	23	22	25	44**
(mg/100 ml.)	G5	26	23	23	23	29**
Serum	H1	.....	.....	369	397	341*
Cl	H2	.....	426	401	451**	370**
(mg/100 ml.)	G5	360	408**	353*	350	291**
Serum	H1	.....	.....	11	12*	12
Ca	H2	.....	12	12	15	13
(mg/100 ml.)	G5	12	12	11	12	12
Serum	H1	.....	.....	14.8	13.7	9.9**
Na: K	H2	.....	15.7	16.9	15.4	9.0**
(ratio)	G5	14.2	14.2	14.6	14.1	9.7**
RBC	H1	.....	.....	6.4	6.1	6.9
($\times 10^6$/mm^3)	H2	.....	7.9	7.6	7.8	10.7**
	G5	8.5	7.1*	7.2	7.0	8.4**
Hemoglobin	H1	.....	.....	7.1	6.7	6.5
(g/100 ml.)	H2	.....	9.8	10.6	10.0	12.6**
	G5	11.1	10.0	8.6**	6.4**	8.4
Hematocrit	H1	.....	.....	29.3	24.8	28.9
(%)	H2	.....	40.3	38.5	34.0**	53.0**
	G5	43.9	36.0	31.0**	26.0**	31.0
Eosinophils	H1	.....	.....	175	373	637**
(per mm.^{3})	H2	.....	176	208	74	448**
	G5	.....	12	150	144	90
Total	H1	.....	.....	4,769	2,810*	2,834**
neutrophils	H2	.....	3,963	3,292	3,453	4,041
(per mm.^{3})	G5	2,392	3,693	1,160**	1,649	1,390
Segmented	H1	.....	.....	3,832	1,646**	2,188
neutrophils	H2	.....	3,391	3,129	3,395	3,913
(per mm.^{3})	G5	2,312	3,583**	1,095**	1,641	1,372

* Significantly different from previous treatments ($P \leqslant 0.05$).
** Highly significantly different from previous treatments ($P \leqslant 0.01$).

the 17.2-18.8 Na : K ratios reported for normal cows and 15.9-16.4 Na : K ratio for normal calves (7, 16). The fall in Serum Na : K to means of 9.0-9.9 during the period of adrenal deficiency was indicative of a decrease in Serum Na, an increase in Serum K, or both. In all cases, the fall in Na : K was highly significant.

Another constant finding in these adrenalectomized calves was the depression of the heart rate and the irregularity of the heart beat in extreme deficiency. This appeared to be correlated with the pronounced increase in serum potassium. It has been reported that a high concentration of potassium salts in the blood causes slowing of the heart and arrhythmia due to a direct effect of potassium on the contractile mechanism of cardiac muscle and on the electrical properties of cardiac fiber membranes (1, 12).

The effect of adrenalectomy on the formed elements of the blood in these three calves was variable and not enough calves were used to draw any definite

conclusions concerning these effects. According to Gordon (9), adrenalectomized rats maintained on 1% saline developed an anemia characterized by a reduction in RBC, hemoglobin, and hematocrit levels. All of these calves exhibited a lower than normal RBC during the period of salt therapy and one (G-5) showed a significant reduction following adrenalectomy. Normal values for calves have been reported as 9.2-9.8 $\times$ 10^6 RBC/cmm, 10.1-11.7 g. hemoglobin/100 ml., and hematocrits ranging from 29.4-43.0% (11, 21). According to these

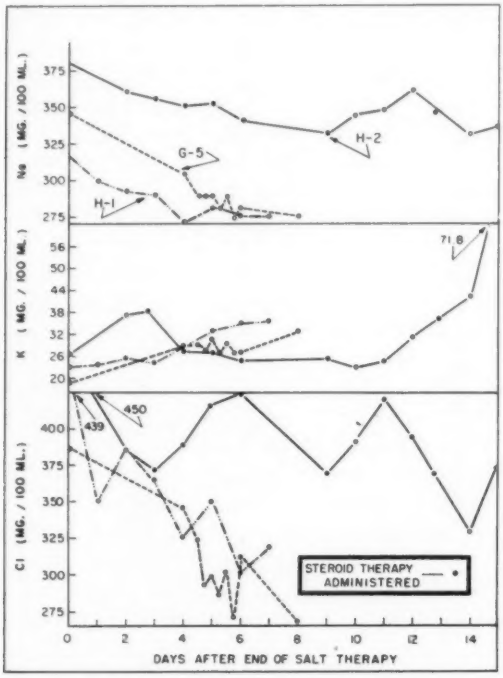


FIG. 1. Changes in blood serum sodium, potassium and chlorides of adrenalectomized calves following withdrawal of steroid (except as noted) and salt therapy.

standards, Calves H-1 and G-5 exhibited a marked anemia throughout the experimental period following adrenalectomy. Calf H-2, though low in RBC, was within the normal range in hemoglobin concentration and hematocrit. Calves H-2 and G-5 exhibited a significant rise in RBC during the adrenal-deficient period, compared to the periods of steroid and salt therapy which were accompanied by a similar increase in hematocrit. This was similar to the increase in RBC, hemoglobin, and packed cell volume described by others (13, 20).

The leukocyte picture also varied greatly in these calves. Two calves, H-1 and H-2, showed a marked eosinophilia during the adrenal-deficient period and G-5 showed insignificant eosinophil changes. Since administration of

cortical steroids (9, 13) or ACTH (9, 17) causes a significant reduction in eosinophils, it is not surprising to find an eosinophilia occurring during adrenal deficiency, though this is not always the case (9). Jones (13) has cited the changes in white blood cells following adrenalectomy and indicates that the most striking increases are in eosinophils and immature neutrophils. The neutrophil picture was variable in the calves in the present study. No significant changes were noted in the lymphocytes or monocytes following adrenalectomy of these calves.

ACKNOWLEDGMENT

The authors wish to express their appreciation to C. N. Graves, L. L. Ewing, and Gene Potter for their technical assistance in this study, and to Drs. R. M. Thomas and E. I. Pilchard of the Diagnostic Laboratory, College of Veterinary Medicine, for the microscopic analysis of the blood samples.

REFERENCES

(1) Brooks, C. M., Hoffman, B. F., and Suckling, E. E. Excitability of the Heart. Grune and Stratton, New York. 1955.

(2) Coffin, D. L. Manual of Veterinary Clinical Pathology. Comstock Publishing Co., Inc., Ithaca, N. Y. 1947.

(3) Cowie, A. T., and Stewart, J. Adrenalectomy in the Goat and Its Effects on the Chemical Constituents of the Blood. J. Endocrinol., 6: 197. 1949.

(4) Cowie, A. T., and Tindal, J. S. Adrenalectomy in the Goat. Replacement Therapy and the Maintenance of Lactation. J. Endocrinol., 16: 403. 1958.

(5) Cupps, P. T., Fowler, M. E., Turk, R. M., and Briggs, J. R. Effects of Adrenalectomy in the Bull. (Abs.) J. Animal Sci., 4: 1319. 1960.

(6) Estergreen, V. L., Jr., and VanDemark, N. L. Some Blood Changes Following Bilateral Adrenalectomy in the Bull Calf. J. Dairy Sci., 42: 926. 1959.

(7) Fisher, E. W. The Concentration of Some of the Inorganic Constituents in the Plasma of Healthy Ayrshire Cattle. Brit. J. Nutrition, 14: 9. 1960.

(8) Goding, J. R., and Denton, D. A. The Effects of Adrenal Insufficiency and Overdosage with DOCA on Bilaterally Adrenalectomized Sheep. Australian J. Exptl. Biol. Med. Sci., 35: 301. 1957.

(9) Gordon, A. S. Endocrine Influences upon the Formed Elements of Blood and Blood Forming Organs. Recent Prog. Hormone Research, 10: 339. 1954.

(10) Holcombe, R. B. Investigations on the Urinary Excretion of "Reducing Corticoids" in Cattle and Sheep. Acta Endocrinol., Suppl. 34. 1957.

(11) Holman, H. H. Changes Associated with Age in the Blood Picture of Calves and Heifers. Brit. Vet. J., 112: 91. 1956.

(12) Howell, W. M. Vagus Inhibition of the Heart in Its Relation to Inorganic Salts of the Blood. Am. J. Physiol., 15: 280. 1906.

(13) Jones, I. C. The Adrenal Cortex. At the University Press, Cambridge. 1957.

(14) Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J. Electrolyte Balance Studies in Adrenalectomized Dogs with Particular Reference to the Excretion of Sodium. J. Exptl. Med., 57: 775. 1933.

(15) Mansson, J., and Obel, N. Technique of Adrenalectomy in the Ruminant. Cornell Vet., 48: 197. 1958.

(16) McSherry, B. J., and Grinyer, I. The pH Values, Carbon Dioxide Content, and the Levels of Sodium, Potassium, Calcium, Chloride, and Inorganic Phosphorus in the Blood Serum of Normal Cattle. Am. J. Vet. Research, 15: 509. 1954.

(17) Merrill, W. G., and Smith, V. R. A Comparison of Some Cellular and Chemical Constituents of Blood at Time of Parturition and After Administration of Adrenocorticotropin. J. Dairy Sci., 37: 546. 1954.

(18) OSTLE, B. Statistics in Research. Iowa State College Press, Ames. 1954.
(19) RICHTER, C. P. Increased Salt Appetite in Adrenalectomized Rats. Am. J. Physiol., 115: 155. 1936.
(20) ROGOFF, J. M., AND STEWART, G. N. Studies on Adrenal Insufficiency in Dogs. II. Blood Studies in Control Animals Not Subjected to Treatment. Am. J. Physiol., 78: 711. 1926.
(21) SCHULTZ, A. B. Influence of Adrenaline Administration on Some Blood Constituents in Holstein Calves. J. Dairy Sci., 42: 166. 1959.
(22) SISSON, S., AND GROSSMAN, J. D. The Anatomy of the Domestic Animals. 4th ed. W. B. Saunders Co., Philadelphia. 1953.
(23) SMOLLICH, A. Gestalt, Topographie, Masse, und Gewichtsverhaltnisse der Nebennieren des Rindes. Anat. Anz., 105: 205. 1958.
(24) WHITEHORN, J. C. A System of Blood Analysis. Supplement II. Simplified Method for the Determination of Chlorides in Blood or Plasma. J. Biol. Chem., 45: 449. 1921.

FACTORS AFFECTING THE SECRETION OF IODINE^{131} INTO MILK OF LACTATING GOATS [1]

E. P. REINEKE

Department of Physiology and Pharmacology, Michigan State University, East Lansing

SUMMARY

Radioactive iodine (I^{131}) was injected into eight lactating goats and its relative concentration in the iodide and protein-bound iodine (PBI) fractions of milk and blood serum was compared. About 92% of the I^{131} in milk occurred as iodide and the remainder was found in the PBI fraction. During 70 hr. following I^{131} injection the iodide concentration in milk of control animals was more than ten times that in blood serum, indicating an active process in mammary tissue for the secretion of iodide. Milk: serum iodide concentration was not affected by administration of 5.0 g. thiouracil or 50.0 mg. of nonradioactive KI daily. The concentration gradient was abolished, however, by injecting 1.0 g. daily of KSCN. None of the drug treatments had a discernible effect on the PBI fraction of milk. Excluding values for the thiocyanate group (where milk iodide secretion was blocked), a 0.795 correlation ($P = 0.07$) was found between milk iodide: serum iodide ratio and daily milk yield. In a preliminary experiment, this correlation was 0.913 ($P = 0.01$). Thus, it is pointed out that in high-producing animals total iodide secretion into milk is increased not only because of the greater volume of milk produced but also because of the higher iodide concentration gradient maintained in highly active mammary tissue. This loss of iodide in milk could indirectly become a limiting factor on milk production by reducing the amount of iodide available for the formation of thyroid hormone.

The secretion of administered I^{131} into milk has been reported in lactating women (6), cows (7), goats (2, 13), dogs (12), rabbits (1), and rats (5, 9, 10). Experiments in several different species (2, 5, 6, 9) show that the level of I^{131} in milk can attain levels many times the amount found concurrently in blood serum, indicating active secretion of this element by mammary tissue. A similar I^{131} concentrating ability has also been shown in lactating rat mammary tissues incubated with I^{131} in vitro (4, 8). Both in vivo (10) and in vitro (8), the response of mammary tissue to goitrogenic drugs parallels that of the thyroid gland.

In an earlier paper (2) it was observed, contrary to expectations, that goats on a minimal iodine intake had a lower thyroid secretion rate than normal controls, together with a reduced thyroidal I^{131} uptake and a high milk: plasma I^{131} ratio. From these observations, it was suggested that milk secretion would tend to become a self-limiting process because of reduced thyroid function resulting from the udder-kidney-thyroid competition for available iodine. The results of further experiments on some of the factors involved in the secretion of I^{131} into milk, and some related observations on thyroidal I^{131} uptake, are included in the present report.

Received for publication January 7, 1961.

[1] Published with the approval of the Director, Michigan Agricultural Experiment Station, as Journal Series No. 2737.

EXPERIMENTAL PROCEDURE

Eight mature dairy goats of mixed breeding that were in their 5th to 6th mo. of lactation were divided into four groups containing two animals each. The goats had access to mixed pasture prior to and during the experiment. They were also fed, according to appetite, a grain mixture consisting of 188 lb. ground corn, 200 lb. ground oats, 92 lb. soybean oil meal, 10 lb. steamed bone meal, and 5 lb. common salt. Experimental treatments were allocated as follows: Group A, control; Group B, 50 mg. NaI injected subcutaneously once daily; Group C, 1.0 g. KSCN in 5.0 ml. of solution injected subcutaneously once daily; Group D, 2.5 g. thiouracil given orally by gelatin capsule each morning and evening. At the beginning of the experiment, each goat was given the first dose of the drug treatment indicated, and immediately thereafter was given 0.5 MC I^{131} [2] by subcutaneous injection.

Separate aliquots were prepared from the injection solution in appropriate amounts to serve as standards for the thyroid counts and the milk and blood serum counts, respectively.

Thyroid radioactivity was measured as described earlier (2), except that 3/8 in. of lead was placed over the face of the scintillation counter to absorb part of the radiation. Without such an absorber the count rate from the thyroids of some animals receiving the heavy dose of I^{131} given in the experiments exceeded the capacity of the counter.

Samples of mixed milk and also of strippings from each morning and evening milking of each goat were taken during 70 hr. following I^{131} injection. Jugular vein blood samples were taken immediately after each milking, allowed to clot, and the serum separated. Four-milliliter samples of milk and blood serum, respectively, were pipetted into counting vials for measurement of total radioactivity. To determine the protein-bound I^{131}, 14 ml. of distilled water and 2 ml. of 10% $ZnSO_4$ were added to 2 ml. of milk or serum in a 50-ml. centrifuge tube. Finally, 2 ml. of 0.5 *N* NaOH was added dropwise, with vigorous shaking. After standing for 20 min. the tubes were centrifuged, the supernatant discarded, and the precipitate washed twice with 2.0-ml. portions of distilled water. The precipitates were transferred, with the aid of KOH solution, to 4-ml. counting vials.

All samples were counted with a scintillation well counter connected to a laboratory scaler. Appropriate standards made from the injection solution were counted at the same time and the results were computed as percentage of dose per liter of milk or blood serum. The I^{131} iodide content was computed as the difference between the whole milk or serum count per liter and the PBI^{131} count per liter. Determinations were done on both the mixed milk samples and the strippings, but inasmuch as no appreciable differences were found, data will be presented for the whole milk samples only.

[2] Obtained from Oak Ridge National Laboratories, Oak Ridge, Tennessee.

RESULTS AND DISCUSSION

The charts in Figure 1 depict the pattern observed for the I^{131}-labeled components in milk and blood serum of one goat from each of the four treatment groups. Since the semilog curves for milk iodide, serum iodide and, in most cases, milk PBI are essentially parallel, the relationships between these components can be described most easily by simple ratios. The ratios between the several I^{131} fractions in serum and milk were computed for each animal at each sampling period and then averaged to give the summary in Table 1.

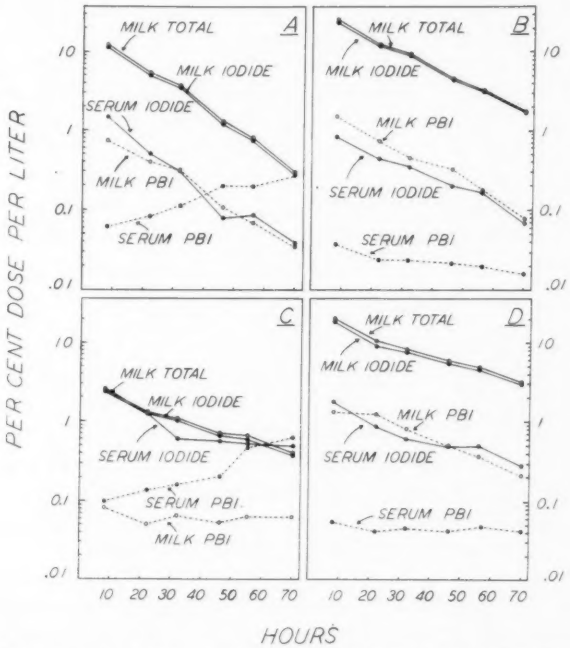


FIG. 1. I^{131} fractions in milk and blood serum of dairy goats. Chart A—Control Goat No. 2; Chart B—Goat No. 3, 50.0 mg. NaI daily; Chart C—Goat No. 5, 1.0 g. KSCN daily; Chart D—Goat No. 8, 5 g. thiouracil daily.

Influence of experimental treatment on thyroidal iodine metabolism. The shape of the serum PBI curve reflects in each case the influence of the experimental treatment on metabolism of iodide by the thyroid gland. It is well established that before entering this fraction, iodine must undergo organic combination in the thyroid and be released to the circulation.

In controls (Chart A) the PBI increased with time, as would be expected normally. Each of the other treatments was selected for its known influence on thyroidal iodine metabolism.

The extra iodide given to Group B was sufficient to flood the thyroid with Nonradioactive I^{127} and consequently prevent organification of administered I^{131}.

TABLE 1

Mean concentration ratios of I^{131} in blood serum and milk during a period of 70 hr. after I^{131} injection

Group and goat No.	Total milk: total serum	Milk iodide: serum iodide	Milk iodide: milk PBI	Mean milk yield per period	Percentage I^{131} dose in milk	70-hr. Thyroid I^{131} retention
				(ml.)		
A. Control						
1	5.7 ±2.54	13.5 ±2.52	5.9 ±1.31	989	27.1	31.5
2	5.7 ±1.39	9.9 ±1.38	11.2 ±1.03	1,052	23.3	36.8
B. 50 Mg. NaI Daily						
3	23.7 ±1.81	24.6 ±1.52	17.3 ±1.21	1,153	67.4	.06
4	1.7 ±0.03	1.6 ±0.55	11.4 ±2.50	448	9.4	.70
C. 1 g. KSCN daily						
5	0.85 ±0.47	1.07 ±0.43	14.0 ±2.95	906	6.4	32.5
6	1.06 ±0.40	1.09 ±0.35	12.9 ±2.76	393	3.7	29.7
D. Thiouracil, 5 g. daily						
7	7.5 ±1.17	10.2 ±1.12	10.8 ±2.00	576	28.5	3.20
8	10.9 ±0.59	10.7 ±0.49	11.3 ±1.07	512	27.1	0.63

± Standard error of the mean.

Thiocyanate, when given in sufficient dosage, will block trapping of iodide by the thyroid (11). In both goats of this group, blockage of the thyroid trapping mechanism was incomplete, as shown by both the increase in serum PBI (Chart C) and the high thyroidal I^{131} uptake (Table 1).

Thiouracil blocks thyroid function by interfering with organic combinations of iodine, but it does not affect the iodide trapping mechanism (3). Its effect is reflected in a low serum PBI (Chart D) as well as a low thyroidal I^{131} retention (Table 1).

I^{131} concentration in milk compared to blood serum. The average milk iodide: PBI ratio for all samples in Table is 11.8, equivalent to about 92% iodide. This compares closely with the iodide level in cows' milk reported by Lengeman and Swanson (7). In the milk of rats (10), up to 86% of the I^{131} was found to be organically bound as monoiodotyrosine, and in dogs up to 60% was protein-bound (12). In both cases, organic binding was blocked or reduced by propyl thiouracil. There is no indication that the proportion of PBI in goat milk was influenced by any of the experimental treatments. The methods employed, however, were probably not sufficiently sensitive to detect changes in the comparatively small milk PBI fraction of this species.

In agreement with earlier reports in the goat (2), dog (12), rat (10), and women (6), the results in Table 1 show that the mammary glands have remark-

able ability to concentrate iodide above the levels prevailing in the blood serum. The two control goats had an average milk iodide : serum iodide ratio of 11.4.

Even though 1.0 g. KSCN (Group C) was insufficient to block the thyroid, it reduced the milk : serum iodide ratio to about one, indicating that the udder is far more sensitive than the thyroid gland to this drug. This result indicates that thiocyanate blocks the active secretory mechanism for iodide in mammary tissue, since the amount appearing in the milk is no more than can be accounted for by simple diffusion. A similar effect of thiocyanate has been found in lactating rat mammary tissue studied in vitro (8).

The milk iodide : serum iodide ratio as well as the percentage of I^{131} dose in milk of the thiouracil-treated goats was similar to that of the controls, even though the thyroidal I^{131} retention was markedly reduced. These results illustrate a clear-cut difference between the mammary and thyroid iodine mechanisms. Once iodine enters mammary tissue it passes into the milk largely as iodide. In the thyroid, the iodide collected initially must pass through the biochemical steps leading to organic combination or it will be returned rapidly to the blood. In Table 1 only the 70-hr. retention of thyroidal I^{131} is given, because peak uptake of I^{131} occurs in normal goats at about this time (2). However, counts taken at 24 and 48 hr. showed that there was a transitory I^{131} uptake by the thyroids of thiouracil-treated animals, in line with the above interpretation.

The effects of iodide supplementation (Group B) on milk iodide concentration appear at first glance to be contradictory, in that both high and low milk iodide : serum iodide ratios were found in the two animals of this group. Furthermore, Goat No. 3, the highest milk producer in the experiment, had the highest iodide concentration ratio. The iodide dose was sufficient in both cases to substantially prevent thyroid accumulation of I^{131}, simply by dilution of the isotope. The percentage of I^{131} dose eliminated in the milk of both animals, compared to the thyroid uptake, shows that the udder has a much greater total capacity than the thyroid for handling iodine.

Inasmuch as the experimental treatments given to Groups A, B, and D did not have a discernible effect on the milk iodide : serum iodide ratio, it seemed valid to pool these values to determine the effect of milk yield on concentration ratio. As determined by least squares, the relation between milk iodide : serum iodide ratio (y) and milk yield in milliliters per period (x) can be expressed by the equation $y = -2.1538 + 0.01897x$. A correlation coefficient of 0.795, significant at the 7.0% level, was found.

In a preliminary experiment conducted with the same goats and treatments 2 mo. earlier, the results paralleled closely those presented here and, therefore, will not be reported in detail. It is of interest, however, that in six animals divided into control, iodide, and thiouracil treatment groups in the preliminary experiment there was a 0.913 correlation between milk yield and milk iodide : serum iodide ratio that was significant at the 1.0% level. Thus, in high-producing animals total iodide secretion into milk is increased not only because of the greater volume of milk produced but also because of the greater iodide con-

centration gradient maintained in highly active mammary tissue. These findings support further the conclusion of Flamboe and Reineke (2) that, at least under conditions of limited iodine supply, lactation tends to become a self-limiting process because of reduced thyroid function resulting from the udder-kidney-thyroid competition for available iodine. The recent report of Grosvenor on experiments with lactating rats (5) is also in accord with this interpretation. If similar relationships are found in dairy cattle this could be a factor of considerable importance in herd management.

ACKNOWLEDGMENT

The author is indebted to Dr. Jack F. Wagner and Mr. H. W. Kaczkofsky for technical assistance.

REFERENCES

(1) Brown, Grant K., and Galton, V. A. Iodinated Compounds in Milk After Radioiodide Administration. Biochim. et Biophys. Acta, 27: 422. 1958.

(2) Flamboe, E. E., and Reineke, E. P. Estimation of Thyroid Secretion Rates in Dairy Goats and Measurement of I^{131} Uptake and Release with Regard to Age, Pregnancy, Lactation, and Season of the Year. J. Animal Sci., 18: 1135. 1959.

(3) Franklin, A. L., Lerner, S. R., and Chaikoff, I. L. The Effect of Thiouracil on the Formation of Thyroxine and Diiodotyrosine by the Thyroid Gland of the Rat with Radio-active Iodine as Indicator. Endocrinology, 34: 265. 1944.

(4) Freinkel, N., and Ingbar, S. H. The Metabolism of I^{131} by Surviving Slices of Rat Mammary Tissue. Endocrinology, 58: 51. 1956.

(5) Grosvenor, Clark E. Secretion of I^{131} into Milk by Lactating Rat Mammary Glands. Am. J. Physiol., 199(3): 419. 1960.

(6) Honour, A. J. Secretion of Radioiodine in Digestive Juices and Milk in Man. Clin. Sci., 11: 447. 1952.

(7) Lengeman, F. W., and Swanson, E. W. A Study of the Secretion of Iodine in Milk of Dairy Cows, Using Daily Oral Dose of I^{131}. J. Dairy Sci., 40: 216. 1957.

(8) Maqsood, M., and Reineke, E. P. In Vitro Uptake of I^{131} by Mammary Tissue. Am. J. Physiol., 199: 829. 1960.

(9) Potter, G. D., and Chaikoff, I. L. Identification of Radioiodine Compounds in Milk of Rats Injected with ^{131}I-Iodide. Biochim. et Biophys. Acta, 21: 400. 1956.

(10) Potter, G. D., Tong, W., and Chaikoff, I. L. The Metabolism of I^{131}-Labeled Iodine, Thyroxine, and Triiodothyronine in the Mammary Gland of the Lactating Rat. J. Biol. Chem., 234(2): 350. 1959.

(11) Vanderlaan, J. E., and Vanderlaan, W. P. The Iodide Concentrating Mechanism of the Rat Thyroid and Its Inhibition by Thiocyanate. Endocrinology, 40: 403. 1947.

(12) VanMiddlesworth, L., and Haney, F. Iodide Secretion in Milk of Dogs. Am. J. Physiol., 179: 681. 1954.

(13) Wright, W. E., Christian, J. E., and Andrews, F. N. The Mammary Elimination of Radioiodine. J. Dairy Sci., 38: 131. 1955.

TECHNICAL NOTES

LIPOLYSIS BY A B-ESTERASE PREPARATION FROM MILK [1]

Milk contains enzymes capable of preferentially hydrolyzing the primary ester positions of the synthetic triglycerides, 2-oleyl dipalmitin and 2-palmitoyl diolein (3). Forster *et al.* (2) have demonstrated that milk also contains an enzyme, B-esterase, which hydrolyzes tributyrin and has been designated as an aliphatic esterase or lipase. The question arose, does B-esterase hydrolyze long-chain triglycerides in the same sequence as milk lipase? The preparation of a B-esterase concentrate from milk (8), by one of the authors (T.L.F.), permitted this question to be answered.

B-esterase concentrate was added at a level of between 1 and 3% to an emulsion of synthetically prepared 2-oleyl dipalmitin (3) in pasteurized skimmilk and the resulting mixture incubated for 30 min. in a water bath at 38° C. with shaking. After extraction and separation of the free fatty acids (3), these were identified as methyl esters by gas-liquid chromatography. (Aerograph Model A-90C.) The concentrations of palmitic and oleic acids were determined from the areas under the peaks on the charts by a disc integrator built into the recorder. In addition, 1 g. of the B-esterase concentrate was mixed with 60 ml. of pasteurized, homogenized milk and incubated at 38° C. with shaking for 30 min. The mixture was extracted and the 1- and 2-monoglyceride contents estimated (5). Acid degree values were determined by the silica gel method (4) and the water-insoluble free fatty acids were recovered qualitatively, methyl esters prepared, and the latter compounds indentified by gas-liquid chromatography. Three trials were conducted with the synthetic triglyceride and one with milk.

[1] This investigation was supported in part by research grant (A-2605) from the National Institute of Arthritis and Metabolic Diseases, Public Health Services.

Preferential hydrolysis of the primary ester positions by the B-esterase preparation is demonstrated by the relatively high molar percentage, 86.2, of palmitic acid in the free fatty acids from lipolyzed 2-oleyl dipalmitin, Table 1. Thus, the B-esterase preparation hydrolyzes 2-oleyl dipalmitin in the same sequence as milk lipases (3). The relatively high proportion of 2-monoglycerides, 81.9%, in the digested milk fat (Table 1) lends further support to preferential hydrolysis of primary ester positions by the B-esterase preparation. Again, similar results have been obtained with milk lipases (6). The presence of diglycerides in the digested milk fat is indicated by the following assumptions: If 2 m*M* of free fatty acid is designated as having been liberated from each millimole of monoglyceride, the resulting quantity, 4.98, from the data in Table 1, is far short of the 10.72 m*M* of free fatty acids recovered. Therefore, if little glycerol is produced, then diglycerides of the 1,2-type must be present (6). The small amount of fatty acid apparently released from the 2-position in 2-oleyl dipalmitin may be due to hydrolysis from the 2-position, impure triglycerides, or acyl migration to the 1-position followed by hydrolysis. Acyl migration is known to occur with both mono- and diglycerides (1, 7) and could account for the 13.8 *M*% oleic acid found in the free fatty acids.

TABLE 1

Lipolysis by a B-esterase concentrate from milk

Substrate	Products of lipolysis						
Oleyl dipalmitin	Free fatty acids—*M* % [a]						
	Palmitic 86.2						
	Oleic 13.8						
Milk fat [b]	Monoglycerides—2.49 m*M*/100 g. fat						
	2-Isomer 81.9%						
	1-Isomer 18.1%						
	Acid degree value [c]						
	10.72						
	Water-insoluble free fatty acids						
Acid	10:0	11:0	12:0	13:0	14:1	14:0	15:0
Wt. %	2.5	0.5	5.1	0.6	1.0	11.9	1.0
Acid	16:1	16:0	17:0	18:2	18:1	18:0	?
Wt. %	3.0	25.4	0.5	1.5	26.9	18.3	1.7

[a] Average of three trials.
[b] One trial.
[c] Silica gel method.

The identity of the fatty acids liberated from milk fat by the B-esterase preparation (Table 1) shows that the concentrate released long-chain fatty acids. No attempt was made to recover the short-chain acids.

These data indicate that B-esterase concentrate contains an enzyme(s) with properties similar to milk lipase, in that both preferentially hydrolyze the primary ester positions of long-chain synthetic triglycerides, both preferentially hydrolyze the primary ester positions of milk triglycerides, and both release long-chain fatty acids from milk triglycerides.

R. G. JENSEN
G. W. GANDER
J. SAMPUGNA
Department of Animal Industries
Storrs Agricultural Experiment Station
Storrs, Connecticut

AND

T. L. FORSTER
Department of Dairy Science
State University of Washington
Pullman, Washington

REFERENCES

(1) CROSSLEY, A., FREEMAN, I. P., HUDSON, B. J. F., AND PIERCE, J. H. Acyl Migration in Diglycerides. J. Chem. Soc., 760. 1959.
(2) FORSTER, T. L., BENDIXEN, H. A., AND MONTGOMERY, M. W. Some Esterases of Cow's Milk. J. Dairy Sci., 42: 1903. 1959.
(3) GANDER, G. W., AND JENSEN, R. G. Specificity of Milk Lipase Toward the Primary Ester Groups of Some Synthetic Triglycerides. J. Dairy Sci., 43: 1762. 1960.
(4) HARPER, W. J., SCHWARTZ, D. P., AND EL HAGARAWY, I. S. A Rapid Silica-Gel Method for Measuring Free Fatty Acids in Milk. J. Dairy Sci., 39: 46. 1956.
(5) JENSEN, R. G., AND MORGAN, M. E. Estimation of the Monoglyceride Content of Milk. J. Dairy Sci., 42: 232. 1959.
(6) JENSEN, R. G., DUTHIE, A. H., GANDER, G. W., AND MORGAN, M. E. Some Evidence Supporting the Specificity of Milk Lipases for the Primary Hydroxyl Positions of Milk Glycerides. J. Dairy Sci., 43: 96. 1960.
(7) MARTIN, J. B. The Equilibrium Between Symmetrical and Unsymmetrical Monoglycerides and Determination of Total Monoglycerides. J. Am. Chem. Soc., 75: 5483. 1953.
(8) MONTGOMERY, M. W., AND FORSTER, T. L. Partial Purification of the B-Esterase of Bovine Milk. J. Dairy Sci., 44: 721. 1961.

INTERESTERIFIED BUTTER OIL

Improvement in the spreadability of butter should enhance its consumer acceptance. Spreadability is influenced by the composition and structure of the fat, the crystalline condition of the fat, and the distribution of moisture and nonfat constituents. In general, these factors can be controlled. However, the chemical composition and structure of the fat can not be controlled economically or technically within the present definition of butter.

The consistency of lard has been improved by rearranging the fat structure by either random (7) or directed interesterification (6), the improvement being greater with the latter method.

Interesterification is conducted in an inert atmosphere, using sodium potassium alloy catalyst. The reaction is random if liquid fats only are present, but directed if the temperature is low enough to allow formation of some solid fat. An inert solvent facilitates directed interesterification (3).

The improvement of lard by interesterification suggested a similar approach for improving butter oil. This note presents the results of such a study.

EXPERIMENTAL PROCEDURES

Several lots of butter oil were prepared by mixing butter with five to seven parts of water at 60° C. and passing the mixture through a separator. The separated oil was mixed with warm water and separated. The oil was thus washed three or four times and dried by heating it at 95-100° C. and 2-5 mm. pressure for 1 hr., with oxygen-free nitrogen bubbling through it to provide agitation.

In preliminary experiments sodium methylate was used as the catalyst and the ratio of solids to liquids was determined by a centrifugal method. The methods finally adapted were found to be superior.

For random interesterification, the butter oil was heated at 40-45° C. for ⅓ to 6 hr. during agitation in a nitrogen atmosphere, using 0.1 to 0.3% sodium potassium alloy catalyst. Shortly after the catalyst was added, an orange color developed which disappeared with reappearance of the original yellow color after the catalyst was discharged by passing into the oil a stream of moist carbon dioxide and adding a very slight excess of acetic acid. The treated oil was washed thoroughly with warm water.

For directed interesterification, the butter oil was treated as above, except as follows: Usually hexane or xylene, 11-23% by weight, was added prior to starting the reaction, in order to facilitate mixing and reaction at lower temperatures (3). The reaction was started at 25-38° C. and the temperature was dropped in

three to five stages, with some mixing at each stage, until the mixture was rather stiff—usually to 10-25° C. The catalyst was discharged, the oil washed, and the solvent removed by distillation.

In experiments 1-DF and 2-DF, interesterification was carried out in a food mixer, without solvent. Some difficulty was experienced in maintaining an inert atmosphere for long periods and, therefore, this procedure was not used further.

Interesterified butter oil was deodorized by passing steam through it for 1 to 2 hr. at 200° C. and 5-10 mm. pressure to remove off-flavor, particularly bitterness.

ANALYSES

An indication of hardness was obtained by using a modification of a micro-penetration method (4) and a precision penetrometer.[1] The fat was tempered 1 hr. before the penetration measurement.

Other characteristics determined were as follows: cooling curve (5), dilatometry (2), iodine value (1), insolubility in hexane at 4° C. (3), solubility in 95% ethanol at 4° C., and capillary melting point.

The cooling curves and the dilatometric and penetration data shown in Figures 1, 2, and 3 are typical of the changes brought about by interesterification.

RESULTS AND DISCUSSION

In Experiment 2-DF, the iodine values before and after interesterification were 30.25 and 30.15, respectively, suggesting no over-all change in percentage of different fatty acids resulting from interesterification.

Directed interesterification brought about greater changes than random interesterification. Increases in melting point were greater in the presence of a solvent than in its absence. The melting point was raised about 15° C. by directed, and only about half as much by random, interesterification. Insolubility in hexane was about 41% with directed interesterification and about 3% with the control. The solubility in 95% ethanol was somewhat greater with directed than with random interesterification.

Increased insolubility in hexane reflects an increase in the quantity of higher melting, long-chain, saturated triglycerides, whereas increased solubility in ethanol reflects an increased quantity of short-chain glycerides.

The cooling curve data in Figure 1 indicate a wide distribution of all fatty acids in the original butter oil. During directed interesterification higher melting fatty acids concentrate on the same glycerides, yielding considerable crystals which melt within a narrow range. This is responsible for the upturn in the cooling curve.

The pattern of percentage of solid fat distribution at different temperatures (Figure 2) was altered slightly by random and considerably by directed interesterification. For the latter, the relatively broad temperature range

[1] A Precision Penetrometer, made by the Precision Manufacturing Company, was used. It is not implied that the U. S. Department of Agriculture recommends this company or its product to the possible exclusion of others in the same business.

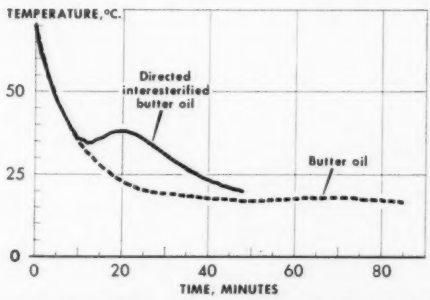


FIG. 1. Cooling curve for butter oil and directed interesterified butter oil. Room constant at 15.6° C.

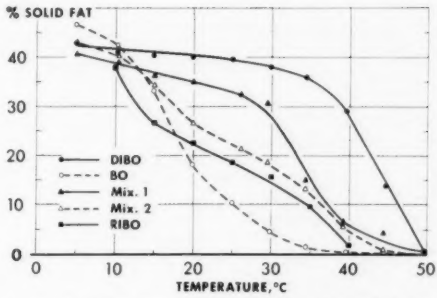


FIG. 2. Per cent solid fat in directed interesterified butter oil (DIBO), butter oil (BO), Mix 1 (one part DIBO and two parts BO), Mix 2 (two parts DIBO and one part BO), and random interesterified butter oil (RIBO).

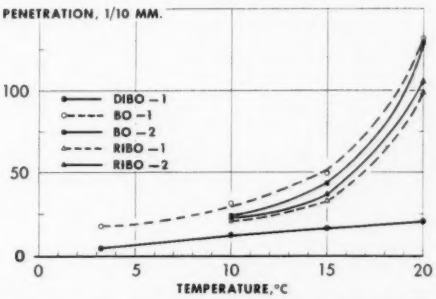


FIG. 3. Penetration data for butter oil (BO), random interesterified butter oil (RIBO), and directed interesterified butter oil (DIBO).

TABLE 1

Interestifying conditions and some properties of butter oil before and after interesterification

Experimental conditions							Properties				
				Temperature							
Expt.	Wt. BO [b]	Cat.	Solv.	Start	Final	Time	M.P.	Hexane	Insol. M.P.	Fat I.V.	Ethanol sol. fat
(No.)	*(g.)*	*(%)*	*(%)*	*(° C.)*	*(° C.)*	*(hr.)*	*(° C.)*	*(% of BO)*			*(%)*
1-R	340	0.3		40	45	⅓	39.7	11.2	54.5	6.35	10.6
							33.5 [a]	3.0	52.1		7.25
2-R	600	0.1		55	55	3	40.6	8.6	53.0	4.87	12.1
							33.6	4.4	52.6	6.26	6.55
3-R	600	0.1		55	55	4	40.7				
							33.6				
4-R	600	0.1		55	55	6	43.3				
							33.6				
1-DF	880	0.48		30	20	3	44.2				
							34.5				
2-DF	841	0.7		30	20	3	44.4				
							34.5				
1-DS	678	0.1	22.9 X	30	10	e	42.5				
							33.5				
2-DS	678	0.1	11.3 X	30	15.6	d	47.5	41.0	55.0	4.15	12.55
							35.5	3.0	52.1		7.25
3-DS	443	0.6	20.3 H	38	25	e	47.4				
							35.6				
4-DS	328	0.3	16.3 H	25	10	f	48.5	41.4	54.0	9.86	14.0
							33.5	3.0	52.1		7.25

[a] Underscored data are for the control butter oil.

[b] Abbreviations: BO = butter oil; Cat. = NaK catalyst, % of BO; D = directed interesterification; F = using food mixer; H = hexane; I.V. = iodine value; M.P. = melting point; R = random interesterification; sol. = soluble; Solv. = solvent; X = xylene.

[c] 182 hr. in five temperature stages.

[d] 159 hr. in four temperature stages.

[e] 40 hr. in three temperature stages.

[f] 61 hr. in five temperature stages.

of 5 to 25° C., where the percentage of solid fat changes but slightly, is noteworthy.

Penetration and dilatometric data (Figures 2 and 3) do not correlate well except within certain limited temperature ranges. Penetration data, an indication of hardness, show a more nearly linear relation with temperature than does the percentage of solids. Therefore, penetration should be of more value than dilatometry in predicting the spreadability of butter made from interesterified butter oil.

Changes in the percentage of solid fat resulting from mixing directed interesterified butter oil and normal butter oil in the ratio of 2 to 1 indicate that in the temperature range 10-30° C., the solid fat is as much as 3% more than the calculated value, but between 35 and 40° C. it is 10-12% less than the calculated value. The latter difference agrees in magnitude with values observed by Woerfel and Bates (8) for mixtures of lard and tallow at 10° C.

Some difficulty was experienced in removing the last traces of xylene. Hence, hexane is the preferred solvent.

An interesterified, steam-deodorized butter oil was recombined with skimmilk. It was scored 37 by a panel of experienced judges of milk quality, which is as good as many market milks.

Pound and other cakes made with steam-deodorized, interesterified butter oil were inferior in flavor and texture to those made with untreated butter oil. Cakes made with steam-deodorized, untreated butter oil were similar in flavor and texture to those made with non-deodorized untreated butter oil.

The properties of interesterified butter oil, as well as mixtures containing one-third to two-thirds butter oil, suggested no application for general use in butter or other foods. However, removal of the higher melting glyceride fraction that separates during directed interesterification should yield a softer fat. The

latter alone, or mixed with ordinary butter oil, should serve to make butter of almost any desired hardness.

HERMAN D. WEIHE
Dairy Products Laboratory
U. S. Department of Agriculture
Eastern Utilization Research and Development Division
Washington, D. C.

REFERENCES

(1) BENHAM, G. H., AND KLEE, L. An Improved Method for Determination of Iodine Numbers. J. Am. Oil Chem. Soc., 27: 127. 1950.
(2) BRAUN, W. Q. Report of the F.A.C. Subcommittee on Dilatometry, 1956-1957. J. Am. Oil Chem. Soc., 34: 344. 1957.
(3) ECKEY, E. W. Directed Interesterification in Glycerides. Ind. Eng. Chem., 40: 1183. 1948.
(4) FEUGE, R. O., AND BAILEY, A. E. Measurement of the Consistency of Plastic Vegetable Fats. A Standard Micropenetration Technique. Oil and Soap, 21: 78. 1944.
(5) LUDDY, F. E., MORRIS, S. G., MAGIDMAN, P., AND RIEMENSCHNEIDER, R. W. Effect of Catalytic Treatment with Sodium Methylate on Glycerine Composition and Properties of Lard and Tallow. J. Am. Oil Chem. Soc., 32: 522. 1955.
(6) PLACEK, C., AND HOLMAN, G. W. Directed Interesterification of Lard. Ind. Eng. Chem., 49: 162. 1957.
(7) VANDER WAL, R. J., AND VAN AKKEREN, L. A. Modified Lard and Process of Producing Same. U. S. Pat. 2,571,315. October 16, 1951.
(8) WOERPEL, J. B., AND BATES, R. W. Blending and Measurement of the Properties of Shortening. Food Technol., 12: 674. 1958.

MILKING EFFICIENCY AND DURABILITY OF MILKING MACHINE INFLATIONS OF VARIOUS COMPOUNDINGS[1]

The idea has been prevalent among dairymen that milking machine inflations of synthetic rubber do not milk as well as natural inflations. Thus, despite a longer useful life for the synthetics, the suspected lower milking efficiency has been a deterrent to their use.

Rate of fat absorption by inflations has been shown (2) to vary markedly with composition of rubber.

This study was initiated in an attempt to learn whether inflations of different compounding would produce different rates of milking. In addition, it was hoped that some conclusions might be reached as to the decline in milking rate that might be encountered with continued usage of the inflations.

METHODS

The milking rate of ten different groups of milking machine inflations were studied with the Colorado State University Holstein-Friesian herd. Milking rate was determined as the percentage of total milk production which was produced in 2 min., as suggested by Beck *et al.* (1). The milking machine inflations were classified in groups according to design and material, as listed in Table 1.

Type III or IIIa always served as the control group, since these inflations were manufactured under rigidly controlled conditions and inflations of the same compounding had been studied extensively in previous work (4). There were nine separate trials or phases of this study involving the comparison of either Type III or IIIa against all the other groups.

In a preliminary trial, the milking rate was established for each cow and on this basis the cows were divided into two as nearly equal groups as possible. The cows were milked in a six-cow elevated milking parlor. All equipment and facilities were less than 1 yr. old and installed and regulated to the latest specifications of the manufacturer (vacuum level, 15 inches of mercury). One group of cows was milked by three units on one side of the parlor and the other group by three units on the other side. Each group consisted of 20 cows.

At the start of each experimental phase, new liners were installed on all milking units. The three units on one side (south) were always equipped initially with the liners of Type III or IIIa, which served as the control or comparison group in all trials. The other side (north) was equipped with one of the other types of liners under study. Following the installation of new liners, the milking rate was determined for six consecutive milkings.

The control (Type III or IIIa) and the experimental group under study were then reversed (control group on the north side and the experimental group on the south side). This reversal procedure was followed once a week during each phase of the study. Milking rates were determined for six consecutive milkings after each reversal. Each trial or phase of this project lasted 3 wk., with the exception of Trial 5, which lasted 8 wk.

Standard procedure was followed in milking the cows. Milk let-down was stimulated by wiping the teats and the floor of the udder for 30 sec. with a paper towel soaked with water containing disinfectant, and dried with another paper towel. This was followed by stripping

[1] Published with the approval of the Director, Colorado Agricultural Experiment Station, as Scientific Series Publication No. 636.

TABLE 1

Design and compounding material of the milking machine inflations studied

Inflation type	Design	Material
I	Tapered Body	Special purpose synthetic compound A
Ia	Straight Body	Special purpose synthetic compound A
II	Tapered Body	Special purpose blend compound B
IIa	Straight Body	Special purpose blend compound B
III	Tapered Body	Rubber compound X
IIIa	Straight Body	Rubber compound X
IV	Straight Body	Neoprene
V	Straight Body	Rubber compound Y
Va	Straight Body	Rubber compound Z
VI	Straight Body	Special purpose synthetic compound C

two or three full hand-squeezes of milk from each quarter into a strip cup. A time interval of approximately 1 min. elapsed between stimulation and application of teat cups.

The interior surfaces of the inflations were inspected periodically for blistering, deterioration, and other possible abnormalities. The inflations were checked periodically for flatness by turning the inflation between the index finger and thumb.

The final trial involved six different types of inflations (Ia, IIa, IIIa, IV, V, and VI), which were installed on the six milking units and inspected once a week as described above. This trial was initiated to study the useful life of the six inflations of the same design but of different compounding. During this trial milking efficiency was not included, since the only purpose was to determine the useful life of each type of inflation.

Temperature and weather data were collected from the Colorado State University Weather Station, to determine whether environmental changes could be related to milking efficiency or to the deterioration of inflations.

RESULTS AND DISCUSSION

Graphic presentation of the average milking rate for the two groups of cows in each of the trials is shown in Figure 1. The control rubber inflations (Type III or IIIa) were compared to six other compounds: two other rubbers (V and Va), two special-purpose synthetics (I and VI), a special-purpose blend (II), and a neoprene (IV). In addition, the study involved differences in design of inflations (straight vs. tapered body) of the same compounding.

There were few differences in milking rate between the various compounds or designs when compared with the control group or with each other. Milking efficiency was not increased when, at the start of a new trial, new inflations were substituted for used inflations. Very little change was observed in the milking efficiency of those inflations (IIIa, IV, V) in which the interior surface did deteriorate with continued usage. These results do not agree with the results obtained by White and Folds (5), from a limited study of milking rate, in which they found neoprene inflations to be slightly superior in milking rate to natural rubber inflations.

Weather and temperature observations taken during the experiment show little relation to milking efficiency except under extreme conditions. In Trial 6, there were 11 in. of snow on the tenth day of the experiment, which may account for the large decrease in milking efficiency on this date.

During Trial 8, unavoidable irregularity in the time of milking produced marked fluctuations in milking efficiency, which were independent of the type of liner used.

Throughout this study, no clear influence of the type or the usage of inflations on the milking efficiency was apparent, either from the average of groups or among individual cows. A brief report (3) indicates that similar results were obtained when six types of natural and synthetic rubber liners were evaluated for milking rate by cine photography. During the same time, fluctuations due to individual differences, stage of lactation, extremes of weather, and regularity of milking were apparent. The conclusion seems warranted that the latter factors are of far greater significance in determining the rate of milking than any of the characteristics of inflations studied in these trials.

In the last trial, each of the various compounded materials was placed on a separate milking machine to study its useful life. The Type IV inflations showed flatness and blistering at the end of 4 wk., while all the other types showed no change. Types IIIa and V inflations began to show blistering at the end of 6 wk. Types Ia, IIa, and VI showed no visible changes or flatness after 8 wk. These observations are the same as those noted during individual comparisons of liner types. White and Folds (5) reported that neoprene consistently outlasted natural rubber inflations under farm conditions. The contrasting results found in this study may be the result of using neoprene of a formulation different from that used by White and Folds (5).

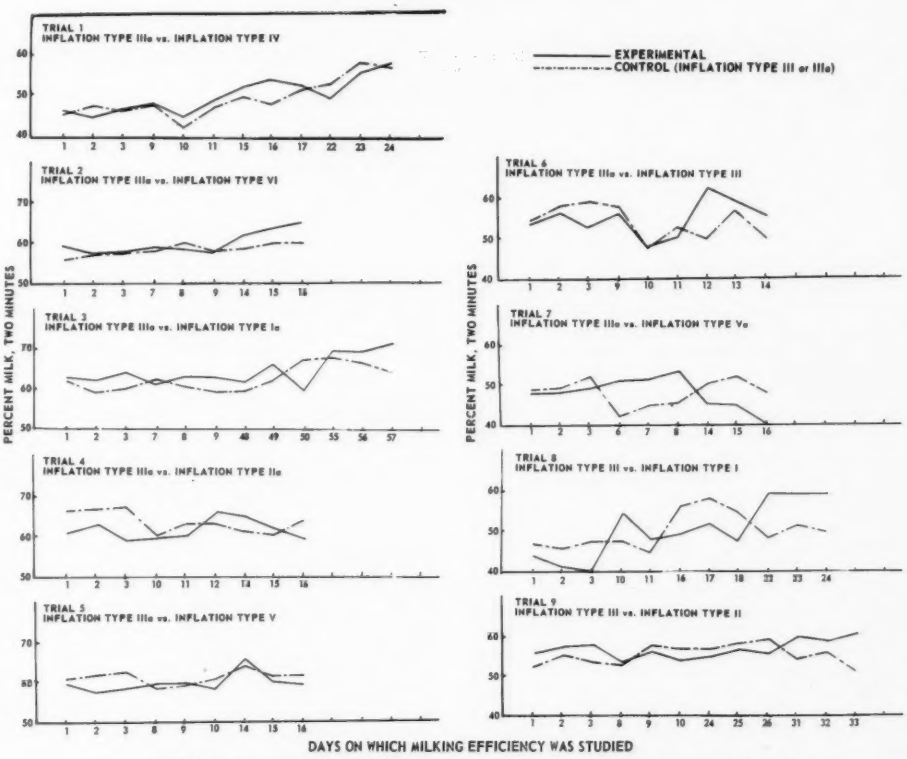

EXPERIMENTAL
CONTROL (INFLATION TYPE III or IIIa)
TRIAL 1
INFLATION TYPE IIIa vs. INFLATION TYPE IV
TRIAL 2
INFLATION TYPE IIIa vs. INFLATION TYPE VI
TRIAL 3
INFLATION TYPE IIIa vs. INFLATION TYPE Ia
TRIAL 4
INFLATION TYPE IIIa vs. INFLATION TYPE IIa
TRIAL 5
INFLATION TYPE IIIa vs. INFLATION TYPE V
TRIAL 6
INFLATION TYPE IIIa vs. INFLATION TYPE III
TRIAL 7
INFLATION TYPE IIIa vs. INFLATION TYPE Va
TRIAL 8
INFLATION TYPE III vs. INFLATION TYPE I
TRIAL 9
INFLATION TYPE III vs. INFLATION TYPE II
PERCENT MILK, TWO MINUTES
DAYS ON WHICH MILKING EFFICIENCY WAS STUDIED

FIG. 1. Milking rate associated with different types of milking machine inflations.

The control inflations (Types III or IIIa) were of the same compounding as the inflations used in a previous investigation at this station (4). At that time, serious blistering of the inner surface occurred after a few days of use during the winter months, while in this study a period of 6 wk. elapsed before blistering was noticeable. The only apparent difference between the two experiments was the type of milking machine used. In the interval between these two experiments the herd had been moved to a new location, but management was essentially the same. Some turnover in cows making up the herd had occurred, but no more than normally occurs in a herd of this size.

GERALD M. WARD
VIRGIL R. ELSEY
HERBERT JORDAN
Colorado State University
Fort Collins
AND
Gates Rubber Company
Denver, Colorado

REFERENCES

(1) BECK, G. H., FRYER, H. C., AND ROARK, DONALD B. Use and Interpretation of Milk Flow Curves in Measuring Variations in the Response of Cows to Machine Milking. J. Dairy Sci., 34: 58. 1951.

(2) CLARKE, P. M., BERRIDGE, N. J., AND GARDNER, E. R. The Deterioration of Milking Rubbers. V. The Effect of Rubber Composition. J. Dairy Research, 22: 144. 1955.

(3) CLOUGH, P. A., DODD, F. H., GARDNER, E. R., AND COPPER, J. The Relationship Between the Properties of the Rubber in Milking Machine Liners and Milking Rate. Rept. Natl. Inst. Research Dairying. p. 26. 1958. (Abstr.)

(4) WARD, G. M., ELSEY, V. R., AND HURRY, J. A. The Effect of Milking Practices upon the Deterioration of Milking Machine Inflations. J. Milk Food Technol., 20: 312. 1953.

(5) WHITE, J. C., AND FOLDS, G. R. A Study of Milking Machine Inflations Made of Neoprene Compared with Those of Natural Rubber. J. Milk Food Technol., 17: 256. 1954.

OUR INDUSTRY TODAY

A COMPARISON OF SCORES AND MEASUREMENTS OF THE UDDER PROPORTIONS

IVAR JOHANSSON[1] AND E. E. ORMISTON
Department of Dairy Science
University of Illinois, Urbana

Shape and attachments of the udder, and size, shape, and placement of the teats are generally considered important characters of the dairy cow, and most breeders try to improve these characters by selection. In the judging of cows, udder and teats are carefully considered and their development and qualities are major factors in the final rating of the animals. On the score-card of the Purebred Dairy Cattle Association, 25 points out of 100 are reserved for the scoring of udder and teats. It is a general experience that when characters of farm animals can be satisfactorily measured, the actual measurements are preferable to scores or other similar evaluations, because the variations are recorded more precisely. This is the situation with regard to weight and wither height as measures of body size, and to milk yield and fat content of the milk. It is true also for the proportions and tilt of the udder and for the size and placement of the teats. Some other udder characters, e.g., attachment to the body, may be impossible to measure, and a system of scoring may, therefore, be necessary.

Benson, Tyler, and Hyatt (1) analyzed data for 83 Ayrshire cows which had been rated semiannually by two official classifiers, one of whom returned for each classification, whereas the other was a different person each time. The repeatability of the ratings made in different years by the same and by different classifiers was as follows: shape and size of udder 0.34 and 0.32, attachment of udder 0.30 and 0.32, veins, teats, and quality of udder 0.37 and 0.30, respectively. The heritability of udder ratings was studied by Freeman and Dunbar (2) on 1,180 dam-daughter pairs of Ayrshire cows. The estimates of heritability were for size and shape of udder 0.08, for udder attachment 0.06, and for veins, teats, and udder quality 0.27. Other estimates of the heritability of scores for udder and teats have been made by Van Vleck, O'Bleness, and Henderson (5), based on 843 dam-daughter pairs of the Holstein-Friesian breed. The results obtained were: for shape of rear udder 0.04, shape of front udder 0.05, udder texture 0.28, depth of udder 0.22, levelness of udder 0.09, strength of attachment of rear udder 0.30, strength of attachment of front udder 0.16, udder quartering 0.18, length of rear teats 0.09, and length of front teats 0.05. The estimates for shape of udder and length of teats are surprisingly low when compared to results that have been obtained on the basis of actual measurements of these characters.

Johansson and Korkman (4) and Johansson (3) studied the udders and teats of some 1,000 cows of the two main Swedish dairy breeds. The udder proportions were determined by weighing the milk from each quarter separately. From the results of the test milkings four udder indices were calculated, viz., (1) I_{FT} = the yield of the two front quarters in per cent of the total yield of the udder; (2) I_{LT} = the yield of the two left quarters in per cent of the total yield; (3) I_F = the yield of the left front quarter in per cent of the total yield of both front quarters; and (4) I_R = the yield of the left rear quarter in per cent of the total yield of both rear quarters. The means ($\bar{x}$) and standard deviations (s) obtained for these udder indices are shown in Table 1.

On an average, the left quarters produced the same amount of milk as did the right quarters, but the yield of the front quarters was only 42.8 and 39.1% of the total yield, respectively, for the two breeds. Furthermore, the standard deviation of the index I_{FT} was about twice as large as that of I_{LT}. There were highly significant differences between daughter groups from different sires and a highly significant dam-daughter correlation (within sires) with regard to I_{FT}, but not for any of the three other indices. The estimates of heritability were 0.76 ± 0.12 for I_{FT} and 0.08 ± 0.08 for I_{LT}. The results show that the proportion of yield between front and rear quarters is highly heritable, whereas the variations between the left and right quarters is almost wholly nongenetic, probably being caused by developmental irregularities and udder infections. Accordingly, the prospects are good for an improvement of the proportion between the front and rear quarters by selection, but there seems to be little possibility for a genetic improvement of the proportions between the left and right quarters.

A comparison was made between the indices I_{FT} and I_{LT} and the scores of the udders assigned by a technical assistant. The agreement was rather poor. By exterior examination it

[1] Present address: Institute for Animal Genetics, Royal Agricultural College, Uppsala, Sweden.

TABLE 1
Averages and standard deviations for the udder indices in two Swedish dairy herds

	No.	Johansson				Johansson and Korkman			
		Per cent of total yield of the udder							
		I_{FT} (front/total)		I_{LT} (left total)		Front index (I_F)		Rear index (I_R)	
		$(\bar{x})$	(s)	$(\bar{x})$	(s)	$(\bar{x})$	(s)	$(\bar{x})$	(s)
Swedish Red and White cattle	569	42.8	5.83	50.0	3.05	49.9	4.38	50.1	3.74
Swedish Friesian	488	39.1	6.59	49.8	3.87	49.3	5.33	50.0	4.60

was possible to point out the more extreme types of disproportionate udders, but moderate deviations in symmetry were overlooked.

To check how accurately an experienced judge of dairy cattle might be able to assess the actual proportions of secretory tissue when scoring the development of the quarters on the basis of their outer appearance, a study was made of 56 cows at the Illinois Agricultural Experiment Station, representing the five major dairy breeds. The yield of each quarter was weighed at two evening milkings, with an interval of about 5 wk. A special milking machine was used with four partitions of the pail, one for each quarter of the udder.[2] Immediately before the test milkings, the udder proportions were scored, using a scale of 1 to 10 for each of the following three items: the proportion between the two front and the two rear quarters, the proportion between the two front quarters, and the proportion between the two rear quarters. The score 5 indicated complete symmetry, whereas lower or higher figures were intended to express the degree of under- or over-development corresponding to actual measurements to the yield (40, 60%, etc.). In estimating the proportions between front and rear udder, half as well as full points were used (4, 4.5, 5.0, 5.5, 6, 6.5, etc.), but for the proportion between left and right quarters of the front and rear udder, respectively, only full points were used. For example, the cow 1628 on the first observation was scored as follows: front half 3.5, rear half 6.5, left front 5, right front 5, left rear 6, right rear 4. These scores indicated that the two front quarters of the udder were estimated to represent 35% and the two rear quarters 65% of the total udder development; the two front quarters were equally developed; and the left rear quarter showed greater development than did the right rear quarter, a ratio of 6:4.

The data were analyzed to estimate the repeatability of measurements and scores, and to show how closely measurements and scores agree, i.e., the correlation between them. To obtain a more direct comparison between measurements and scores, each measurement was transferred to a grade, based on the percentage figure; e.g., 42.6-47.5% of the milk produced by the front quarters was translated to grade 4.5, etc. Since it may be assumed that the errors of measurements and scores are larger for low- than for high-producing cows, the data were first divided into two groups, and the error of measurement, defined as the square root of the variance within cows, was calculated for each group separately. The differences between the two groups were small and insignificant and, therefore, only the results for the total number of cows are presented in Table 2 and no comparison between breeds was attempted.

The repeatability is high for the indices, which are based on actual measurements of the yield of the individual quarters. When the indices are transformed to grades, some of the precision is lost, but in spite thereof the repeatability of the grades is almost twice as high as that of the scores. All the three sets of scores have a low repeatability, but the symmetry of the front quarters seems to be par-

[2] The four-quartered milker unit was courteously placed at the authors' disposal by the DeLaval Separator Company, Chicago.

TABLE 2
Errors of measurement and repeatability of indices, grades, and scores

	Indices		Grades		Scores	
	Error of measurement	Repeatability	Error of measurement	Repeatability	Error of measurement	Repeatability
Front quarters	1.84	0.86	0.25	0.75	0.69	0.33
Rear quarters	1.47	0.89	0.23	0.73	0.55	0.49
Front/total	1.54	0.90	0.22	0.82	0.55	0.47

ticularly difficult to assess. It should be noted that only the relative development of the quarters, and no other udder characteristics, was considered in the scoring of the udder proportions.

The results of the correlation analysis are shown in Table 3.

TABLE 3

Coefficients of correlation between udder indices and scores (within sets of measurements, degrees of freedom 108)

	Index-score	Grade-score
Front quarters	0.83	0.76
Rear quarters	0.65	0.61
Front/total	0.32	0.33

All the coefficients are significant at the 0.1% level. However, the correlation between the score and the index for front/rear symmetry is rather low, which indicates that this character was not assessed with satisfactory accuracy by judging. The accuracy in scoring for symmetry of the front quarters, and the rear quarters, is higher, but these scores and indices are of little value to the breeder from a selection point of view because variation in symmetry of quarter of the udder is almost wholly nongenetic.

It is not surprising that the estimates of heritability of scores for shape of udder and size of teats which have been obtained by Freeman and Dunbar (2) and by Van Vleck, O'Bleness, and Henderson (5) do not deviate significantly from zero. In the first place, the accuracy in evaluating these characters is low, and secondly, the score is often a composite expression for several characters which should be evaluated separately, e.g., shape and size of the udder; or veins, teats, and quality. Johansson (3) found that actual measurements of the teats and their placement had a high or moderately high heritability. The following estimates were obtained: length of teats 0.98 $\pm$ 0.20; diameter of the teats 0.38 $\pm$ 0.22, distance between the front and rear teats 0.50 $\pm$ 0.22, and index of teat placement (the distance between the rear teats in per cent of the distance between the front teats 0.36 $\pm$ 0.22). A selection based on actual measurements of the front/rear symmetry of the udder and of the size and placement of the teats can be expected to yield satisfactory results, whereas a selection based on scores for these characters probably would lead nowhere.

SUMMARY

Actual measurements of the udder proportions, based on the amount of milk produced by the individual quarters, have been compared to the scores for symmetry assigned by an experienced judge of dairy cattle. Each of 56 cows was tested and scored at two evening milkings with about 5 wk. interval. The repeatability of the scores was only half, or less, of that of the corresponding indices based on the yield of the quarters. The correlation between the index I_{FT}, stating the amount of milk from the two front quarters in per cent of the total yield, and the corresponding score was only 0.32. Earlier investigations show that the heritability of the index I_{FT} is rather high (about 0.75). Selection for better balance between front and rear halves of the udder would, therefore, be expected to give good results when based on actual measurements of the milk yield, but would probably be ineffective when based on scores obtained by palpation and visual estimates.

REFERENCES

(1) Benson, R. H., Tyler, W. J., and Hyatt, G. Some Causes of Variation in Type Ratings of Ayrshire Cows. J. Dairy Sci., 34: 502. 1951.

(2) Freeman, A. E., and Dunbar, R. S. Genetic Analysis of Components of Type, Conformation, and Production in Ayrshire Cows. J. Dairy Sci., 38: 259. 1955.

(3) Johansson, I. Untersuchungen über die Variation in der Euter- und Strichform der Kühe. Z. Tierzücht. Züchtungsbiol., 70: 233. 1957.

(4) Johansson, I., and Korkman, N. Heritability of Udder Proportions in Dairy Cows. Hereditas, 38: 131. 1952.

(5) Van Vleck, L. D., O'Bleness, G. V., and Henderson, C. R. Heritabilities of Type Appraisal Traits and Their Genetic Correlations with Production. J. Dairy Sci., 42: 925. 1959.

CONTAINER PROBLEMS IN REFRIGERATED WAREHOUSES [1]

W. A. Maclinn

The Refrigeration Research Foundation, 12 North Meade Avenue
Colorado Springs, Colorado

The Refrigeration Research Foundation became interested in container problems in refrigerated warehouses approximately 2 yr. ago. We became interested in the problem at the request of the refrigerated warehousing industry. So that you will understand why we were called upon for help, following is a brief résumé of The Foundation's role in the food field.

Several years ago, members of the refriger-

[1] Presented at the Dairy Manufactures Conference, University of Wisconsin, Madison, 1961.

ated warehousing industry decided to provide means to stimulate scientific investigations in the field of food refrigeration. Since this industry consisted of many relatively small companies, they planned to achieve together those objectives that large companies achieve individually through research—such as improved technology, sound development, better service to customers and the public generally, and an advanced body of new scientific fact that would sustain the refrigeration industry in more remote years.

To this end they founded The Refrigeration Research Foundation. It is a tax-exempt, nonprofit corporation, incorporated under the laws of the State of Illinois, and it has been established for 16 yr. Approximately 140 companies financially support The Foundation—food processors, refrigerated warehouse companies, and transportation organizations.

The Foundation does not do research. It supports the research of educational institutions and other research bodies whose field of investigation is along the lines of the potential development of the refrigerated warehousing industry. The results of this research are then interpreted by The Foundation and its advisors into practical handling procedures in the warehouse.

The practical handling procedures are designed to provide the conditions of handling perishable commodities with the least possible damage to quality. The field of research and subsequent handling is not limited to foods, but does go into other fields such as rubber, furs, hides, batteries of all types, and any item that can lose its value by haphazard holding conditions.

The Foundation is supervised by a Board of Governors numbering 28, of whom 21 represent industry and seven represent the public interest. The Honorary Chairman of the Board, who was Chairman of the Board for 15 yr., is Dr. Samuel Cate Prescott, Emeritus Dean of Science, the Massachusetts Institute of Technology in Cambridge, Massachusetts. The Chairman of the Board is Dr. Louis B. Howard, Dean and Director of the Illinois Agricultural Experiment Station, Urbana, Illinois. The Foundation's research program is guided by a group of 21 outstanding scientists in the United States and Canada.

In line with the request for help on packaging problems, The Foundation made a nation-wide survey to determine if package failures were truly a problem in the refrigerated warehousing industry, or were merely isolated cases caused by mishandling by individual companies. The survey showed that package fatigue and failures were actually an economic problem for all warehouses.

Following this, a Packaging Symposium was held in Chicago, led by our Scientific Advisory Council, to which package engineers and designers were invited. In attendance were 24 representatives of the packaging industry, 15 from the warehousing industry, seven food packers, five from the trade publication field, and four, other than our scientists, from the educational field.

At the close of the sessions, it was concluded that new warehouse handling methods, environment within refrigerated rooms, and foods themselves were causing unusual stresses and strains on packages. The packaging representatives at the Symposium indicated that protection from fatigue and failure could be built into the containers, as the packaging industry learned how to do this. However, it was concluded that these stresses and strains were not being considered when specifications were being drawn up by the food manufacturer. It was proposed then that The Foundation seek the opportunity to acquaint food manufacturers with the information presented at the Symposium.

This is why I was delighted to be invited to participate in this Wisconsin Dairy Manufacturers' Conference. I hope you will understand that I am not trying to pin the blame for package failures in refrigerated warehouses on the dairy manufacturers; I am not. I wish only to bring to you a report on the changes that have occurred in the refrigerated warehousing industry over the past few years that have resulted in new stresses and strains on packages in storage.

First, property evaluations have risen and the cost of land for buildings has resulted in the necessity of going to taller structures. Construction costs have necessitated going to higher ceilings and fewer floors. All of this means higher stacking.

Increased labor costs have resulted in some mechanization in warehouses. Hand stacking is gone. Hand-pushed, four-wheel trucks have been replaced by fork lifts moving at 6, 7, or 8 mi. per hour.

Certainly, it is not expected that the individual containers have enough built-in strength to support all of the weight of four pallet loads of produce. Stacking irons are used to strengthen the support. However, it is expected that there should be enough built-in strength to hold up under the load of cases on one pallet.

The fatigue and failure of some packages is not due entirely to excess weight put on them. Until a few years ago, much of the material put into refrigerated warehouses went in storage for several months and then moved out in a body. Today, all storage rooms are being filled, partially emptied, refilled, etc., every few days. Inventories are small and turnover is great.

With the increased movement in and out of refrigerated rooms, there are marked fluctuations in the relative humidities of these rooms. Warm air and commodities coming into the cold rooms give up their moisture in the form

of condensation—sometimes invisible—on the cold containers already in the room. As these packages are made of wood pulp and sometimes rags are mixed in, they are an organic fibrous material. The fibers are natural capillaries and the surface moisture diffuses through these capillaries along a moisture gradient to the point of concentration of moisture. Thus, the moisture in high humidity rooms rapidly moves into the packaging fibers.

These capillaries were shown to have different moisture holding properties, as demonstrated by the moisture content of kraft paper, tobacco, and wood, held at various relative humidities sufficiently long to reach equilibrium.

Relative humidity	Moisture content		
	Kraft paper	Wood	Tobacco
(%)	*(%)*	*(%)*	*(%)*
10	3.0	3.0	5.4
30	5.7	....	11.0
50	7.6	9.3	16.0
70	10.5	....	25.0
90	14.9	22.0	50.0

Organic fibers change in length with their moisture content. The higher the moisture, the longer the fibers and, in general, the fibers soften as they lengthen and thus lose their strength. Stresses and strains occur in container walls with changes of relative humidity in a refrigerated room. These changes in relative humidity cause the fibers to pick up or give up some of their moisture. With this change goes lengthening and contraction of the fibers and, if done enough times, this can result in structural failure. Moisture-resistant barriers are needed to prevent this water vapor absorption by the packages.

Moisture-resistant barriers are usually a coating of some material, plastic or wax or other material, that covers all outer surfaces of the packages. These are all effective barriers to the penetration of moisture. To date, however, they have all been slippery when moist at cool temperatures. To prevent their sliding off or squeezing out of stacks, it is necessary to stipple or otherwise roughen the stacking surfaces. This is sometimes forgotten in the design or specification for the containers, and thus piles fail and accidents happen.

Square containers are a problem. They do not lock together. Therefore, it is necessary to strap them with heavy rope or metal band, each tier, to prevent toppling. This is an added cost of material, plus labor.

It is not my intention to imply that refrigerated warehousemen are without blame for some package failures. They are to blame when they handle containers roughly. They sometimes stack too high, without sufficient aiding supports. However, they can do a better job of handling your products if the containers are—

1. Oblong in shape
2. Nonmoisture-absorbing
3. Nonslipping
4. Have structural strength to support reasonable heights of stacking.

ASSOCIATION AFFAIRS

THE AWARDS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION: A REVIEW

G. M. TROUT, Historian
The American Dairy Science Association

"No endeavor is in vain;
Its reward is in the doing,
And the rapture of pursuing
Is the prize the vanquished gain."

Despite the poetic touch of Longfellow, the American Dairy Science Association recognizes services and achievements of its members by special awards given at the annual meetings of the Association. Starting from a mere suggestion 29 yr. ago, that the society recognize distinctive service of its members, the system of special recognition now encompasses at least eight areas.

The awards have created more-than-usual interest in research, teaching, and extension, both in dairy production and manufactures, as well as in service to the Association itself. The awards program has become the high light of the annual meeting.

Since the idea of special recognition was first conceived, membership in the Association has increased tenfold and attendance at the annual meetings sixfold. Conceivably, many members are not familiar with the origin of the awards and the conditions under which they are granted. With possible enlightenment and encouragement foremost in mind, this historical review of the Association awards is undertaken. The several awards are reviewed herein in the order in which they were approved by the Executive Board.

THE AMERICAN DAIRY SCIENCE ASSOCIATION AWARD OF HONOR

So many facets are reflected in the evolution of the American Dairy Science Association's Award of Honor, the highest award of the Association, that one must look at the brush marks to see the full picture.

The American Dairy Science Association Award of Honor has undergone many changes since its conception in 1932. Actually, the award today is not quite the same as those originally made, but the basic concept of paying special tribute to one of its members for distinctive service remains the same. The evolution of this award is of interest.

(a) *Special tributes.* For the first 26 years in the life of the Association, little thought was given to honoring members who had rendered distinctive service to the Association and to the dairy industry. At the 27th annual meeting, University of Kentucky, Lexington, J. B. Fitch and C. E. Wylie spoke in favor of the society so honoring some of its members. Apparently, the idea caught on, for the following year at the University of Illinois, Urbana, the society paid special tribute in the form of a scroll to Wilber J. Fraser, founding father of the National Association of Dairy Instructors and Investigators (later The American Dairy Science Association). The year following, at Cornell University, Ithaca, a like tribute was tendered Otto F. Hunziker.

At the 1934 meeting, J. M. Sherman presented the following motion which, after much discussion, was adopted:

> "That a permanent committee be appointed to pass on honors confered on individuals at annual meetings; that this be a standing committee consisting of the last three presidents and that the committee make its report to the Board of Directors."

In 1935, at the 30th annual meeting, University of Minnesota, St. Paul, the society presented Martin Mortensen, Iowa, with the third scroll, designated as honorary tribute. Each scroll contained a specific citation to the man being honored. For five years no further membership awards were made. Then, in 1940 at the 35th annual meeting, Purdue University, West Lafayette, the organization honored Julius H. Frandsen with the last special tribute it was to bestow. Not until 1946 was a system of honoring specific members for distinctive service restored and organized under a Committee on Honors.

(b) *Honorary charter life memberships.* Prior to 1946, the Executive Board bestowed honorary life memberships on four charter members. These were: in 1942, Otto F. Hunziker, and in 1944, Wilber J. Fraser, E. S. Guthrie, and C. C. Hayden. Later, in 1956, by interim action of the Executive Board, a special Charter Honorary Membership was bestowed on each living charter member of the Association not heretofore so honored. Receiving this recognition were C. F. Doane, Carl E. Lee, and Charles Thom. These memberships carried all the rights and privileges of the regular Honorary Memberships instituted in 1946 and were awarded to pay extra tribute to those men for their contributions to the early life of the Association.

(c) *Association life memberships.* Also, in 1956, when the Executive Board was acting favorably in honoring three charter members, the Board approved the report of the Membership Committee, which recommended in part

that the Association recognize worthy life members in more increasing numbers if and when applications are made to the Board. Previously, at the 1944, Columbus, Ohio, 38th annual meeting, the Executive Board approved the granting of life membership without payment of dues, upon Board action, to those who had been members of the Association for a period of 25 years or more, immediately before retirement.

At the 53rd annual meeting, Raleigh, 1958, the Board voted changes in the By-Laws which had been approved previously by the membership relative to honorary and life memberships. Accordingly, upon application by or on behalf of the individual concerned, the Association through the Executive Board may grant the title of Life Member to active members who

(a) retire after 25 or more years of Association membership, or
(b) have paid membership dues, including Student Affiliate, for 40 years.

Retirement meant withdrawal from full-time employment in dairy science or in the dairy industry.

To those eligible, the Association Life Membership Certificates were given. In addition, each life member was to receive gratis the JOURNAL OF DAIRY SCIENCE as long as he lived. The list of members granted life membership to date is as follows:

1944	E. G. Hastings		J. A. Newlander
	Chris Larsen		G. W. Wilster
	L. A. Rogers	1959	J. C. Knott
1945	Oscar Erf		F. H. Herzer
	George C. White		C. J. Schneider
1947	S. I. Bechdel		W. M. Regan
	H. A. Harding		J. V. Quigley
	C. W. Larson		T. A. Baker
	H. E. Ross		J. L. Hileman
1948	Robert S. Breed	1960	T. V. Armstrong
1949	O. C. Cunningham		P. T. Dix Arnold
1952	C. Albert Altwegg		A. H. Bayer
1953	C. W. Holdaway		G. B. Caine
1955	H. E. Otting		V. O. Dreyer
1956	A. J. Cramer		T. W. Gullickson
	B. W. Hammer		P. S. Lucas
	L. A. Maynard		E. J. Perry
1957	E. C. Thompson		A. H. Robertson
	J. O. Tretsven		H. A. Ross
	A. L. Darnell		A. W. Rudnick
1958	Harry B. Alger		P. H. Tracy
	W. F. Jones	1961	A. C. Ragsdale
	J. P. LaMaster		C. A. Hutton
	Louis W. Morley		W. H. Martin
			S. J. Brownell

(d) *Association Honorary Membership.* At the 40th annual meeting, Chicago, 1945, the Executive Board approved the report of the Committee on Honors or Special Recognition which finally established principles that might govern the selection of future members to be honored. The committee, consisting of A. C. Dahlberg, H. P. Davis, and H. F. Judkins, chairman, pointed out that heretofore there appeared to be no uniformity as to the award of the two classifications previously used; namely, life membership and honorary life membership. After defining specifically the category of life memberships, the committee recommended:

> "That a classification of honorary membership be established by the American Dairy Science Association and that the qualifications for this membership shall be as follows:
>
> 1. The person selected as an honorary member shall have been a member of the Association for not less than ten years, shall have participated in the activities of the Association, and shall have rendered meritorious service to the dairy industry through contributions in research, teaching, extension, administration, or commercial scientific work.
> 2. No more than one such honor is to be conferred during any one year.
> 3. Any member of the Association may nominate a candidate for honorary membership, such nomination being sent to the Chairman of the Committee on Honors, or the Secretary or President of the Association. The Committee on Honors shall consider all nominations and decide who, if anyone, shall be recommended to the Executive Board as an honorary member. Such recommendation by the Committee shall be made to the Secretary at least six weeks prior to the date of the annual meeting of the Association."

Accordingly, at the annual meeting of the Association, the honorary member is awarded the Association's Certificate of Honorary Membership, with the announcement that during his lifetime he will continue as an active member of the Association with all rights and privileges, including receiving the JOURNAL OF DAIRY SCIENCE, without the payment of further dues.

The interest in this award manifest at awarding ceremonies bespeaks the wisdom of the Committee in defining and clarifying the provision for this honor. Although specifically designated as Honorary Member, the award has been presented variously in its 14-yr. existence as award of honor, dairyman of the year, distinguished service award, and honor award, as well as by its correct name, Honorary Membership. Those members who have been awarded the Association's Certificate of Honorary Membership are:

1946	O. E. Reed	1953	H. P. Davis
1947	R. B. Stoltz	1954	J. B. Fitch
1948	A. A. Borland	1955	W. A. Wentworth
1949	H. B. Ellenberger	1956	H. F. Judkins
1950	M. J. Prucha	1957	Earl Weaver
1951	L. A. Rogers	1958	A. C. Dahlberg
1952	C. L. Roadhouse		

(e) *The Award of Honor.* At the 54th annual meeting, University of Illinois, Urbana, 1959, the Executive Board voted to confirm the interim actions of the Board, one item of which involved changing Article III of the By-Laws so that honorary membership becomes award of honor. The award of honor may be

bestowed upon any person who has been a member of the Association for not less than 25 years and who has made a distinguished contribution to the Association. This interim action made possible presentation of the first award of honor at the 1959 awards program.

The new terminology connotes a distinction not entirely incorporated in the previous designation. Coincidentally, H. P. Davis in presenting to Ollie E. Reed, 1946, the first honorary membership apparently misspoke himself or was 13 years ahead of his time when he referred to the award as an award of honor. The members who have received honorary memberships as award of honor under the new designation are:

1959 J. A. Nelson 1960 W. E. Petersen

Fifty-year citations

At the Golden Jubilee meeting at the University of Connecticut, Storrs, 1956, the Executive Board presented citations on plaques to the five living charter members of the Association. These citations were bestowed upon C. F. Doane, Carl Lee, Otto F. Hunziker, C. C. Hayden, and E. S. Guthrie. E. S. Guthrie was present to receive the plaque which read:

To
E. S. GUTHRIE
CHARTER MEMBER
American Dairy Science Association

In appreciation of unselfish devotion and loyalty to 50 years of Dairy Science
A.D.S.A.
Golden Anniversary
1906-1956

At this meeting, also, tribute was paid Julius H. Frandsen, originator and first editor of the JOURNAL. The citation on the plaque read as follows:

To
JULIUS H. FRANDSEN
FOUNDER AND FIRST EDITOR

Journal of Dairy Science
1917

In grateful recognition of his service to Dairy Science in America

A.D.S.A.
Golden Anniversary
1906-1956

Also, at the Jubilee meeting, the Board voted to honor the Association's Historian, G. M. Trout, for his efforts in preparation of the 125-page document and reduced version (J. Dairy Sci., 39:625-650. 1956) of the history of the Association. This recognition was in the form of a framed, illuminated Award of Merit certificate, signed by the Association's President and Secretary.

THE BORDEN AWARDS

Aside from the special recognitions to members by the Association itself, the Borden Awards in dairy production and in dairy manufacturing were the first of a series of monetary awards to be administered by the American Dairy Science Association. These $1,000 cash and gold medal awards were conceived by W. A. Wentworth, then Public Relations Director, The Borden Company, New York. In presenting the first Borden Awards at the 32nd Annual Meeting, University of Nebraska, Lincoln, 1937, Mr. Wentworth said, in part:

> "Eighty years ago, Gail Borden founded what has now become The Borden Company ....
>
> "In recognition of the high value of research past, present, and future, and in perpetuating the ideals of its founder, The Borden Company sought some fitting manner in which research in dairying might be further stimulated and at the same time modestly rewarded. The American Dairy Science Association accepted the offer to administer two Borden Awards. One of these for most outstanding research work in dairy manufacturing and one for similar work in dairy production."

In the fall of 1936, members of the American Dairy Science Association were startled, if not fascinated, when The Borden Company announced that it would present six prizes or annual awards of $1,000 each in cash and a gold medal (the 2½-in. medal weighs 100 g. and is of 10-k. gold) for meritorious work in science as it is related to the dairy industry. Two of these were to be administered by the American Dairy Science Association and one each by the American Chemical Society, American Association for the Advancement of Science, American Home Economics Association, and the American Public Health Association. As of 1960, the originally designated administrative scientific groups have been changed and the annual awards increased to nine, administered by the following societies:

The American Dairy Science Association
  Dairy Production
  Dairy Manufacturing
The American Chemical Society
The American Academy of Pediatrics
The Association of American Medical Colleges
The American Home Economics Association
The American Institute of Nutrition
The Poultry Science Association
The American Veterinary Medical Association

R. R. Graves, President of the Association, 1937, and R. B. Stoltz, Secretary, lost no time following the announcement of the Borden Awards in securing favorable Board action and setting machinery in motion to administer the awards. Having had no previous experience or rules in administering such awards, the Association was guided only by the judgments of the appointed candidate nominating and se-

lecting committeemen. A letter and nomination blank to members and a special announcement in the February, 1937, JOURNAL invited members of the Associations to make nominations for these awards to the Secretary not later than March 1. Any living citizen of the United States or Canada, of either sex, with no limitations as to age, was eligible to receive these awards. Nominations were to be made for those whose work had been completed and published during the five-year period ending December 31, 1936. However, limitations of the fields and scope of research in making the nominations were pointed out:

> "Since Borden awards are also being made for meritorious work in basic science as related to, or as it affects the Dairy Industry; for work in basic research on vitamins or other nutritional aspects of milk products; for work in the practical application of the findings in nutritional research; and for work in public health as related to the Milk Industry, these awards to be administered by other scientific societies, these phases of research work as applied to the dairy industry should not be considered in making your nominations for research in the production and the processing fields."

As other societies had experienced difficulty in securing nominations from their members, special nomination committees, consisting of the three more recent chairmen of the Production and Manufacturing Sections, were named to receive nominations which had been submitted to the Secretary and to scan the field for eligible candidates. Accordingly, the nominating committee for the Borden Award in dairy production consisted of F. W. Atkeson, K. S. Morrow, and C. Y. Cannon. That for dairy manufacturing consisted of P. H. Tracy, L. M. Thurston, and M. J. Mack.

The selection of the first awards committees was made with considered judgment. Three qualifications were deemed essential, namely: (a) experience in evaluating research, (b) knowledge of problems of the industry, and (c) prestige, that their final selections would remain unquestioned. Thus, the first Borden Award selection committee in dairy manufacturing was named as follows: H. A. Ruehe, H. L. Russell, and O. F. Hunziker. That in dairy production consisted of H. B. Ellenberger, C. W. Larson, and E. G. Woodward.

At 11 A.M. on June 25, 1937, Lincoln, Nebraska, members and friends gathered for the presentation of the first of the Borden Awards. The auspicious moment had arrived. President R. R. Graves gave a brief account of the establishment of the awards, the basic rules for qualification, and the special tasks of the committees. Then Chairmen Ellenberger and Hunziker announced the first Borden Award winners: Carl F. Huffman, Michigan, and Lore A. Rogers, United States Department of Agriculture, production and manufacturing, respectively. Both men responded with prepared papers giving the salient points of their research. These addresses, excellent as they were, were to be the first and last required of the Borden awardees. In the 23 double Borden Award presentations since then, few selections have been so generally acclaimed as those of 1937.

Before the Board of Directors (later the Executive Board) closed their final 1937 business session, the Borden Award rules were discussed, changed, and submitted for further suggestions and approval. This was the beginning of a series of changes yet to be made.

The first significant change in the Borden Award rules became effective in 1938 when a 40-year age limit was imposed upon eligible candidates. This rule was operative for two years, 1938 and 1939. Action of the Board of Directors, Columbus, 1938, created a committee of three to draw up rules for the Borden Award. Accordingly, Harold Macy, who had been asked previously to make a study of the rules, H. A. Ruehe, and F. B. Morrison were appointed to this study committee. Their excellent report, specifying eligibility, nominations, composition, and duties of the nominating and award committees (J. Dairy Sci., 22: 664-666. 1939) was adopted.

In this report the age limit was dropped, but consideration of the work published or completed during the five-year period immediately preceding January 1 of the year during which the award was to have been made was retained. However, under special circumstances, consideration also might be given to related work accomplished by the nominee during a period not to exceed ten years previous to the year the award is to be made. In effect, this provision extended the research consideration period from five to ten years. In 1956, the Executive Board approved the report of the Awards Procedure Review Committe, O. F. Garrett, Chairman, making consideration of the ten-year period of research mandatory. Item 8 of the report read as follows: "Be judged on the basis of work done during the 10-yr. period immediately preceding nomination."

At the 1940 meeting, W. Lafayette, the Secretary, was instructed to enclose with the membership letter and ballots a suitable nomination blank, with brief rules, for the Borden Award. The effectiveness of this method of securing nominations caused the Board at the Burlington meeting, 1941, to abolish the nominating committee for the Borden Awards. Not until the 50th Annual Meeting, East Lansing, 1955, 14 yr. later, did the Executive Board amend the rules "to allow for an easier selection of the recipients." Evidently, this action provided for reinstatement of nominating committees of three each, which again were in operation in 1956 but not in 1957.

At the 46th Annual Meeting, Knoxville, 1951, the Executive Board voted to increase the Bor-

den Award Committees from three to five "to better facilitate the choosing of the recipients of the Borden Foundation Awards." Already, the Borden Award Committee in dairy manufacturing had had this provision in effect for the current selection.

In 1957, the committees consisted of six members each, five of whom were voting members under the administration of the chairman, the nonvoting sixth member. At the annual meeting that year, Stillwater, the Executive Board directed the Vice-President to work with the Awards Committee on mechanics of awards rules procedure. The next year at Raleigh the Board voted that if in the opinion of the committee chairman worthy candidates had not been nominated, he might nominate such individuals and solicit the supporting dossier on behalf of those nominees. The Board voted also, that with the exception of the chairmen the names of the Award Committees not be divulged. Thereafter, only the chairmen of the Award Committees were listed among the officers of the Association.

At the 54th Annual Meeting, Illinois, 1959, the Board authorized the President to appoint a committee to study and develop a plan for preparing dossier material for the several awards.

Board action at the 1960, Logan, meeting removed the element of retirement concerning eligibility of members on award selection committees and asked the Policy Committee to study the limits of the areas of interests in the field of awards, specifying the number and kinds of awards within and without that area of interest.

Borden Award Recipients. The list of Borden Award winners in dairy manufacturing and in dairy production by date and affiliation at the time the award was given is as follows:

(a) Dairy Production Award

1937 Carl F. Huffman, Michigan State University
1938 William E. Krauss, Ohio Agr. Expt. Sta. and Ohio State Univ.
1939 Ralph E. Hodgson, Bureau Dairy Industry, USDA
1940 Charles W. Turner, University of Missouri
1941 Edwin B. Hart, University of Wisconsin
1942 William E. Petersen, University of Minnesota
1943 Lane A. Moore, University of Maryland
1944 Paul H. Phillips, University of Wisconsin
1945 Glenn W. Salisbury, Cornell University
1946 E. Paul Reineke, Michigan State University
1947 Joseph C. Shaw, University of Maryland
1948 Walter L. Gaines, University of Illinois
1949 George H. Wise, Iowa State University
1950 Samuel Brody, University of Missouri
1951 John K. Loosli, Cornell University
1952 John Wm. Hibbs, Ohio Agr. Expt. Sta.
1953 Raymond B. Becker, University of Florida
1954 Lester E. Casida, University of Wisconsin
1955 Clifford W. Duncan, Michigan State University
1956 Harry A. Herman, National Association of Artificial Breeders
1957 J. Thomas Reid, Cornell University
1958 Jay L. Lush, Iowa State University
1959 Noland L. VanDemark, University of Illinois
1960 Norman L. Jacobson, Iowa State University

(b) Dairy Manufacturing Award

1937 Lore A. Rogers, Bureau Dairy Industry, USDA
1938 Kenneth G. Weckel, University of Wisconsin
1939 Stewart L. Tuckey, University of Illinois
1940 Bernard W. Hammer, Iowa State University
1941 Paul F. Sharp, Cornell University
1942 Hugo H. Sommer, University of Wisconsin
1943 Byron H. Webb, Bureau Dairy Industry, USDA
1944 Arthur C. Dahlberg, Cornell University
1945 G. Malcolm Trout, Michigan State University
1946 Paul H. Tracy, University of Illinois
1947 Chester D. Dahle, Pennsylvania State University
1948 Egerton G. Hood, Department of Agriculture, Canada
1949 Francis J. Doan, Pennsylvania State University
1950 Walter V. Price, University of Wisconsin
1951 Samuel T. Coulter, University of Minnesota
1952 Emerson W. Bird, Iowa State University
1953 F. Eugene Nelson, Iowa State University
1954 Paul R. Elliker, Oregon State College
1955 Frank V. Kosikowski, Cornell University
1956 Ernest O. Herreid, University of Illinois
1957 N. P. Tarassuk, University of California
1958 W. James Harper, The Ohio State University
1959 Marvin L. Speck, North Carolina State College
1960 Mark Keeney, University of Maryland

The Borden Award in the Chemistry of Milk administered by the American Chemical Society. Closely allied to dairy manufacturing, but specifically different on the basis for which

the award is made, are the Borden Awards in the chemistry of milk. These awards for "outstanding research in the chemistry of milk and dairy products or in fundamental research that contributes directly and materially to the knowledge of milk and dairy products" have been presented yearly, beginning in 1939. Since the field is closely associated with dairy manufacturing, and since many of the recipients are leaders in that field, the Borden awardees in the chemistry of milk are hereby listed for the information of those not familiar with this area of recognition in dairy research. The Borden Award recipients administered by the American Chemical Society are:

1939 Leroy S. Palmer, University of Minnesota
1941 Claude S. Hudson, U. S. Public Health Service
1942 George E. Holm, Bureau of Dairy Industry, USDA
1943 Earle O. Whittier, Bureau of Dairy Industry, USDA
1944 William Mansfield Clark, The Johns Hopkins University
1945 Ben H. Nicolet, Bureau of Dairy Industry, USDA
1946 Ira A. Gould, University of Maryland
1947 George C. Supplee, G. C. Supplee Research Corp.
1948 Barbour L. Herrington, Cornell University
1949 George R. Greenbank, Bureau of Dairy Industry, USDA
1950 George A. Richardson, Oregon State College
1951 Thomas L. McMeekin, Eastern Regional Research Lab., USDA
1952 Carrell H. Whitnah, Kansas State University
1953 Robert Jenness, University of Minnesota
1954 Donald V. Josephson, Pennsylvania State University
1955 Fred Hillig, U. S. Food and Drug Administration
1956 Sam R. Hoover, Eastern Utilization Research Branch, USDA
1957 Stuart Patton, Pennsylvania State University
1958 William G. Gordon, Eastern Regional Research Lab., USDA
1959 Charles A. Zittle, Eastern Regional Research Lab., USDA
1960 Eugene L. Jack, University of California

THE AMERICAN FEED MANUFACTURERS' ASSOCIATION AWARD

At the 42nd annual meeting of the American Dairy Science Association, Guelph, 1947, the Executive Board "accepted the proposal of the American Feed Manufacturers' Association to make a $1,000 award for the best paper published on animal nutrition, and authorized the President to appoint a committee to draw up rules for handling this award." President Tracy appointed C. F. Huffman, Michigan, Chairman, J. G. Archibald, Massachusetts, and R. B. Becker, Florida, to draw up rules for handling the American Feed Manufacturers' Association Award.

The committee established rules, received nominations, and made selection of the first awardee, George H. Wise, 1948. Only publications during 1946 and 1947 were eligible for consideration in making the selection. Four areas were considered in evaluating the nominee's work, namely: (a) original research; (b) proper presentation; (c) value in dairy cattle nutrition; and (d) the possibility of practical use to the dairy cattle industry. On the basis, only the papers published during the two-year periods preceding the year in which the award was made were considered in making the selections from 1948 to 1956, inclusive. Beginning with the 1957 selection, and continuing to the present, publications of the previous three-year periods were considered. The published results of the research submitted for consideration are judged according to the following score-card:

CRITERIA	POINTS
Originality	25
Design, thoroughness and completeness of investigation	30
Presentation of work	15
Value to dairy production	30
Total	100

The rotating three-man selection committee system established in 1947 prevailed until 1956, when the committee was enlarged to five members. The next year the committee was increased to six members. In 1958, the Executive Board voted that thereafter only the name of the chairman of the committee be divulged.

The names of the American Feed Manufacturers' Association Award winners and affiliations at the time the award was made are as follows:

1948 George H. Wise, Iowa State University
1949 Thomas S. Sutton, Ohio State University
1950 J. Thomas Reid, Cornell University
1951 Thor W. Gulickson, University of Minnesota
1952 Hamilton D. Eaton, University of Connecticut
1953 J. William Thomas, Bureau of Dairy Industry, USDA
1954 Carl F. Huffman, Michigan State University
1955 Norman L. Jacobson and Robert S. Allen, Iowa State University
1956 Joseph C. Shaw, University of Maryland
1957 Earle E. Bartley, Kansas State College
1958 Charles A. Lassiter, Michigan State University
1959 Harry R. Conrad, Ohio Agricultural Experiment Station
1960 W. A. Hardison, Virginia Polytechnic Institute

THE DELAVAL DAIRY EXTENSION AWARD

At the 44th annual meeting of the American Dairy Science Association, Minnesota, 1949, the Executive Board went on record as favoring some sort of award for extension personnel, should a suitable donor be found. Accordingly, the Extension Dairyman Award Committee

presented a plan at the 1950 Cornell meeting which the Board adopted. A suitable donor, the DeLaval Separator Company, had been secured. The Award Committee, consisting of W. E. Wintermeyer, Washington, D. C., Chairman, J. B. Fitch, Minnesota, H. W. Gregory, Indiana, H. O. Henderson, West Virginia, and C. W. Reaves, Florida, selected Enos J. Perry, New Jersey, as the candidate to receive the first DeLaval Achievement Award in Dairy Extension.

The DeLaval Dairy Extension Award is now in its 11th year of operation. The basic requirements for consideration for this award are: the nominee must (a) be a member of the American Dairy Science Association; (b) be actively engaged in full-time dairy extension work at time of nomination; (c) have had a minimum of ten years of dairy extension work in connection with agricultural colleges and state (provincial) and federal extension services; (d) not have received previously a comparable award from the American Dairy Science Association or another administering association based upon the same work; and (e) have made a valuable and noteworthy contribution to the dairy industry.

The recipients are selected on the basis of (a) the extent of public acceptance of their extension program; (b) the effect of their program on the dairy economy and welfare of farm people; (c) qualities of leadership; and (d) training and experience prior to entering the dairy extension fiefild. The award consists of $1,000 and an inscribed certificate.

Those receiving the DeLaval Dairy Extension Award are as follows:

1951	Enos J. Perry, Rutgers University
1952	Stanley J. Brownell, Cornell University
1953	G. E. Gordon, University of California
1954	Charles R. Gearhart, Pennsylvania State University
1955	Floyd J. Arnold, Iowa State University
1956	J. O. Tretsven, Montana State College
1957	Harold R. Searles, University of Minnesota
1958	Lyman H. Rich, Utah State University
1959	Ray Albrectsen, Cornell University
1960	George M. Werner, University of Wisconsin

THE AMERICAN DAIRY SCIENCE ASSOCIATION TEACHING AWARD

Although the American Dairy Science Association was founded, 1906, as the National Association of Dairy Instructors and Investigators, and although teaching had played a very important role in the development of dairy science, no specific recognitions had been given its distinguished teachers. Executive Board action was taken at the 47th annual meeting, Davis, 1952, to appoint a committee "to study the feasibility and mechanics of an Outstanding Teacher Award."

The next year, at Madison, S. M. Salisbury gave a progress report for the Dairy Science Teaching Award Study Committee which the Board accepted as a progress report. The following year, 1954, Pennsylvania, specific recommendations concerning the teacher's award were made to the Executive Board by the enlarged teaching awards committee composed of C. E. Wylie, Chairman, H. P. Davis, H. B. Henderson, R. J. Werner, A. H. Rishoi, and W. B. Combs. These recommendations were to the effect that (a) The American Dairy Science Association give special recognition to outstanding teachers of dairy science; (b) the first recognition to be made at the annual meeting in 1955; (c) a special rotating committee of five be appointed by the President to carry out the details of this award; (d) nominations for this award to be made by members of the Association; (e) there be two awards—one in dairy manufacturing and one in dairy production, according to the sponsorship; and (f) an announcement of the details and other information in regard to this award be made in the JOURNAL OF DAIRY SCIENCE.

The Board voted favorably to take steps to follow the course of action as outlined in the teaching award report. By interim action, the Board approved the Milk Industry Foundation sponsorship of a teaching award to be administered by the Association. Thus, the first teaching award of the American Dairy Science Association, in dairy manufactures, consisting of $1,000 and an appropriately engraved plaque sponsored by the Milk Industry Foundation, was given to Frederick H. Herzer, Mississippi, at the 50th annual meeting, East Lansing, 1955.

The committee recommended that a Production Teaching Award be made in 1956, that one or two members be added to the committee, and that efforts be directed toward securing a sponsor for this award. Such a sponsor was found in the National Dairy Products Corporation. In 1956, Connecticut, the first teaching award in dairy production was presented to William E. Petersen, Minnesota.

Consideration was given at the 55th annual meeting, Logan, 1960, to give a teaching award in dairy manufacturing and one in dairy production the same year, but action was taken not to change the method of recognizing outstanding teachers at that time.

Nominees for the Dairy Science Teaching Award of the American Dairy Science Association must

(a) Have demonstrated outstanding ability as a teacher of dairy science.
  (1) In the odd-numbered years he shall be a teacher primarily in the field of dairy manufacturing.
  (2) In the even-numbered years he shall be a teacher primarily in the field of dairy production.
(b) Be a member in good standing of the American Dairy Science Association.
(c) Be an active teacher at the time of the nomination and have been an active teacher for a period of not less than ten years.

(d) Not have previously received a Teaching Award nor have received another comparable award from the American Dairy Science Association or another administering association based upon the same field of work.

(e) Be judged as a teacher on the following basis:

(1) The impression he has made upon his students by the mastery of his subject, his technique, vision, and attitudes.

(2) The content and nature of the courses he has taught, with emphasis placed upon his responsibilities, organization of teaching materials, original contributions to teaching, and ability to translate new research into terms of useful practice.

(3) His activities as a student counselor, coach of judging teams, in the training of students and placing them in positions for which by natural endowments they seem best suited, and in research (not essential as a factor in consideration for the Award).

(4) His service to industry.

The teaching award recipients to date are:

1955 Frederick H. Herzer, Mississippi State College (Manufactures)
1956 William E. Petersen, University of Minnesota (Production)
1957 G. Malcolm Trout, Michigan State University (Manufactures)
1958 Harry O. Henderson, West Virginia University (Production)
-959 H. A. Bendixen, Washington State College (Manufactures)
1960 Earl Weaver, Michigan State University (Production)

RECOGNITION OF OUTSTANDING PERSONS

The Executive Board in session, Knoxville, 1951, "voted to recommend to the membership that the President, on request of an Annual Meeting host institution, appoint a committee of three to consider the recognition of any outstanding person who might be suggested by the host institution, for recognition at the Annual Banquet. This committee to function for one year only. Further, that the Association go on record as favoring such recognition of outstanding persons."

With this authority, the host institution, the University of Tennessee, on behalf of the American Dairy Science Association, recognized at the awards program the former Governor of Tennessee and Congressman, James McCord, who had done much to promote dairying in Tennessee. A picture of the dairy building, McCord Hall, bearing the following inscription was presented to the Honorable Mr. McCord:

To the Honorable Jim McCord, honor guest of the American Dairy Science Association, in recognition of distinguished service to the dairy industry, June 7, 1951.

THE PAUL-LEWIS LABORATORIES AWARD IN CHEESE RESEARCH

The Paul-Lewis Laboratories Award to recognize and encourage research pertaining to cheese was approved by the Executive Board at the 53rd annual meeting, Raleigh, 1958. The award consists of $1,000 and an appropriately inscribed plaque. Only the published research findings on cheese not heretofore considered for a similar award were to be considered for the Paul-Lewis Laboratories Award in cheese research. The nominee must be a member of the American Dairy Science Association. The first recipient for this award was Walter V. Price, Wisconsin, 1959. The list of the winners is as follows:

1959 Walter V. Price, University of Wisconsin
1960 Frank V. Kosikowski, Cornell University

STUDENT AFFILIATE CHAPTER AWARDS

The American Dairy Science Association Student Affiliate Committee composed of W. L. Slatter, Chairman, L. K. Crowe, I. I. Peters, P. M. Reaves, and W. W. Snyder recommended at the 50th annual meeting, East Lansing, 1955, that some form of recognition be considered for the chapter(s) having the most outstanding activities program. Likewise, the possibility of having an outstanding student award was discussed also.

The following year, 1956, Connecticut, the Board approved the request of the Student Affiliate Committee that winners of outstanding club activity exhibits at the current meeting be granted suitable certificates of merit. The winning student branches were:

1956 Ohio State University
Virginia Polytechnic Institute
University of Minnesota
Rutgers University
Louisiana State University
1957 Virginia Polytechnic Institute
University of Minnesota
University of Georgia
1958 Ohio State University
University of Minnesota
Virginia Polytechnic Institute
Kansas State College
Mississippi State College
Michigan State University
Louisiana State University

Later, Illinois, 1958, through interim action, the Board approved a cash award of $100 and plaque from Association funds for the most outstanding student chapter for 1959. The next year the Board approved continuance of the award. The winners to date are as follows:

1959 Louisiana State University
1960 Virginia Polytechnic Institute

GRADUATE STUDENT SCIENTIFIC PAPER PRESENTATION AWARD

The Student Affiliate Committee, W. L. Slatter, Chairman, L. K. Crowe, L. R. Dowd, P. M. Reaves, and W. W. Snyder, first reported to

the Executive Board at the Golden Anniversary meeting at Storrs, 1956, as one of its suggested projects "a technical paper writing and presentation competition for graduate students." The Board accepted the report with respect to this award and authorized further study on the matter. The special committee appointed to investigate the various aspects of this proposed award, consisting of Glenn Salisbury, W. V. Price, and I. A. Gould, made a progress report at Stillwater, 1957, and a final report at Raleigh, 1958.

In 1958, Raleigh, the Executive Board approved the recommendation of the special study committee for this award and referred the matter back to the Student Affiliate Committee advising possible implementation of such an award, on a trial basis in one section, at the 1959 annual meeting. Further, the Board authorized a $100 prize with a suitable plaque or certificate to the winner, if the committee proceeded with the plan.

Accordingly, competition for this award became effective at the 54th annual meeting, Urbana, 1959. The winner was J. B. Stone, Cornell University, who was presented $100 and a plaque by W. W. Snyder, Chairman of the Student Affiliate Committee. Honorable mention certificates were presented to K. E. Ebner, University of Illinois, and E. W. Wickersham, Pennsylvania State University.

This program was so well received that the Board, in 1959, recommended its continuation and expansion to include the manufacturing section in 1960, the Student Affiliate Committee working out the matter of awards and donors. Again, $100 cash prizes and plaques were presented the winners. Those graduate students winning the Scientific Paper Presentation Award to date are as follows:

1958	J. B. Stone, Cornell University (Production)
1959	J. T. Huber, Iowa State University (Production) John A. Jaynes, Michigan State University (Manufacturing)

EX-PRESIDENT PLAQUE

At the 55th annual meeting, Logan, 1960, the Executive Board voted to present an appropriate plaque at the Awards Ceremony to all future Presidents upon leaving that office and to all living Past-Presidents. Such plaques were presented by mail to the following Past-Presidents, December, 1960, listed according to their term of office and the institution with which they were affiliated at the time:

4th	1912, 1913	J. H. Frandsen, University of Idaho
11th	1925, 1926	O. E. Reed, Michigan State University
12th	1927	J. B. Fitch, Kansas State University
15th	1931	H. B. Ellenberger, University of Vermont
16th	1932	E. L. Anthony, Michigan State University
17th	1933	H. C. Jackson, University of Wisconsin
19th	1935	C. L. Roadhouse, University of California
21st	1937	R. R. Graves, U. S. Department of Agriculture
22nd	1938	H. W. Gregory, Purdue University
23rd	1939	Earl Weaver, Michigan State University
24th	1940	E. S. Guthrie, Cornell University
26th	1942	H. F. Judkins, National Dairy Products Corporation
27th	1943	H. P. Davis, University of Nebraska
28th	1944	A. C. Dahlberg, Cornell University
29th	1945	A. C. Ragsdale, University of Missouri
30th	1946	J. A. Nelson, Montana State College
31st	1947	Fordyce Ely, Ohio State University
32nd	1948	P. H. Tracy, University of Illinois
33rd	1949	W. E. Petersen, University of Minnesota
34th	1950	G. M. Trout, Michigan State University
35th	1951	R. B. Becker, University of Florida
36th	1952	H. A. Bendixen, Washington State College
37th	1953	H. B. Henderson, University of Georgia
38th	1954	W. V. Price, University of Wisconsin
39th	1955	L. A. Moore, U. S. Department of Agriculture
40th	1956	I. A. Gould, Ohio State University
41st	1957	C. F. Huffman, Michigan State University
42nd	1958	D. V. Josephson, Pennsylvania State University
43rd	1959	K. L. Turk, Cornell University
44th	1960	A. C. Fay, H. P. Hood and Sons

DISTINGUISHED SERVICE AWARD

The idea of initiating the American Dairy Science Association Distinguished Service Award secured the approval of the Executive Board at the 54th annual meeting, Urbana, 1959. The Board stipulated that (a) the award be limited to one or more; (b) the recipients be limited to those engaged in commercial work, public welfare, and regulatory activities relating to the dairy industry; and (c) the recipients may or may not be members of the Association. Thereupon, a committee appointed by the President consisted of J. Hoffman Erb, The Borden Company, Chairman, L. A. Moore, Beltsville, H. B. Henderson, Georgia, and H. F. Judkins, ex-officio, reported back to the Association at the 55th, 1960 meeting, Logan. Here the Board approved the proposed rules with

amendments. A six-member, five-voting selection committee was appointed, looking forward to the granting of the first award at the 1961 meeting.

The announcements and specifications for this award (J. Dairy Sci., 43:1539-1540. 1960) include 15 suggested areas of endeavor as a guide to the members making nominations. Without a doubt, the American Dairy Science Association earnestly desires to recognize individuals who have rendered distinguished service in the dairy industry, either in production or processing. In no way does this award overlap or shadow the Association's Award of Honor presented annually to one of its own members.

FOREIGN AWARDS

Aside from Canadian membership, members of foreign countries have been ineligible for consideration for the awards of the American Dairy Science Association, the chief reason being difficulty anticipated or encountered in implementing the nominations and selections according to schedule. Being aware of increased foreign membership and greater attendance of foreign members at the annual meeting, the Board at the 55th annual meeting, Logan, 1960, directed the President to explore the attitude of donors of cash awards to members in foreign countries, should such members be selected for specific awards.

AWARDS SELECTION COMMITTEES

With the exception of the chairman, names of the members of all monetary and honor award selection committees of the American Dairy Science Association are not divulged. The chairmen administer the work of the committee, but are denied the right to ballot for a candidate. This power is reserved for the remaining five men on each of the committees. The committee men are appointed on a rotating-term-of-office basis by the President of the Association. Former awardees may or may not automatically serve a term of office on some committee by routine appointment following receipt of the award. As of 1961, seven separate incognito committees serve the following areas:

(1) Borden Award in dairy manufacturing
(2) Borden Award in dairy production
(3) American Feed Manufacturers Award
(4) DeLaval Extension Award
(5) Dairy Science Teaching Award
(6) Paul-Lewis Laboratories Award for Research in Cheese
(7) Association Award of Honor

SUMMARY

Starting with a mere concept in 1932, that the American Dairy Science Association should recognize in a special way some of its members to which the Association seemed indebted, the idea of special awards now encompasses several areas and embraces many individuals.

Difficulty is encountered in attempting to place values on the several awards of the American Dairy Science Association beyond their specific monetary and intrinsic values to the recipients. Naturally, those signally honored in some way or another have experienced a glow of satisfaction accompanied by a deeper sense of loyalty to the Association.

Beyond the shadow of a doubt, the various awards administered and/or given by the Association have kindled a desire of achievement in the breast of every individual member. This, in itself, is beyond price. Such incentives can not be evaluated.

The membership as a whole has come to look forward to the awards-night ceremonies, anticipating the pleasure of sharing the honors with the recipients. In fact, the gathering of associates, friends, and relatives with a kindred spirit at the awards event does much to cement the membership into a living, forward-moving scientific body.

While 196 members have been recognized officially by the Association since 1933, some several times, more than 2,500 members have been grateful that their Association, years ago, took action to recognize merit and service among its own members.

The awards of the American Dairy Science Association would seem to have been a strengthening force for the good of the Association beyond the dreams of those who conceived the several awards and recognitions—some over a quarter of a century ago.

RESEARCH AND EDUCATION ARE THE KEYS TO AGRICULTURAL PROGRESS

R. E. HODGSON

Animal Husbandry Research Division, Agricultural Research Service, USDA
Beltsville, Maryland [1]

We Americans have the privilege of enjoying the most abundant, varied, nutritious, and safe food supply in the world. This food is available to us at a lower cost than it is for most any other people in terms of the labor income required to purchase it. We have ample quantities of the needed foods, so that all of our

[1] Presented at the annual meeting, Southern Division, American Dairy Science Association, Jackson, Mississippi, February 9, 1961.

people could have the ingredients of a truly balanced diet. This is not to say, however, that all of our people presently are consuming an adequate diet.

The American farmer has provided this abundant, high-quality food supply while at the same time utilizing a steadily declining percentage of the nation's working force and while sharing in a proportionately decreasing percentage of the national income.

All of this has been possible because farmers have been introducing revolutionary changes in their methods of operations, which have resulted in increased yields and in increased efficiency of production. The dairy farmer has been no less active in these areas than his neighbors producing other farm commodities.

This past year the average citizen consumed 1,488 lb. of food, according to U. S. Department of Agriculture statistics. Of this amount, more than 420 lb. were dairy products, the largest single food item in terms of bulk weight. Fruits and vegetables ranked next, with 409 lb. One can readily see what a tremendous stake the dairy industry has in feeding the people. This is all the more important when one observes the steady decline in the per-capita use of milk and milk products in recent years. The challenge is before this industry to hold its present enviable position in providing the food supply of the people.

Farmers have been able to achieve these great accomplishments up to the present, but what is in prospect for the future? Conservative estimates indicate that this nation's population will double within the next 50 yr. To maintain present food standards (and they can be improved on), this country will then need twice as much food as is used today. Farmers will have to double their production—crops, milk, and livestock—and they will have to do this with little or no more land and probably with considerably less manpower. There is no question that this goal can be met. But how will it be done? Will we be able to hold the great livestock industries, which provide us with abundant high-quality milk, meat, and eggs, or will our people have to resort to a diet of less animal food products? Agricultural land will have to produce more abundantly and livestock production, including dairying, will have to be much more efficient. Let me repeat, this can be done.

Agriculture, and particularly dairying, as they exist at the beginning of this critical year of 1961, are products of research and the educational application of research findings. America's farmers have made such tremendous progress in increasing production because good information on production, processing, and marketing techniques has been available and they have used it, first to meet a war crisis and secondly to try to improve their competitive position in society. To meet the goals and the challenges of the future, availability of the right kind of information and its proper use will determine in large measure the progress that will be made.

Where did the information come from on which past farming accomplishments have been made? The answer, obviously, is in very large measure the public-supported agricultural experiment stations and the educational institutions, including the Extension Services, both state and federal. Where will the informational resources for the future come from? From these same institutions and, to an increasing extent, also from private research institutions.

It is obvious, then, that so far as agriculture is concerned, the agricultural research and educational institutions have been in the past, and will continue to be even more so in the future, a great national asset and an indispensable investment.

The public agricultural research and extension programs are a large and closely knit cooperative program between the U. S. Department of Agriculture and the land-grant colleges and state universities. The land-grant institutions have the additional duty of training students for careers in agriculture, in agricultural research and education, and related fields. To meet the responsibilities before us, the people, and especially agricultural people, must see to it that proper attention is given to develop the programs of research and education in these institutions, so that they can adequately provide the services needed to promote the kind of agricultural industries we should have. We must also awaken in the nonagricultural public their sense of a responsibility to support research and education, to sustain a prosperous agriculture.

These programs are expensive. For agricultural research alone in 1959, the appropriations amounted to some $238 million, $124 million of which was appropriated by the Congress. Of this, about $92 million is used by the Department of Agriculture research agencies and $32 million is federal grant money to the states. The Department employs some 4,800 scientists, including specialists in the biological, physical, and social sciences, who work at some 300 locations. The state experiment stations employ some 8,400 scientists, some of whom engage in teaching and extension work as well as in research. The research work program is organized in some 15,500 individual projects carried on throughout the United States.

There are some 14,700 agricultural extension workers, of whom about 2,500 are specialists working at the state level. The appropriations in support of this work amount to $150 million, of which $56.7 million was appropriated by Congress and the remainder comes from state and county sources. The number of teachers engaged in agricultural instruction is not available at this time. Many of them engage in research or extension work as well.

The agricultural research and educational

programs are made up of physical, biological, and human resources. Of these, the human resources are by far the most important. The physical resources—laboratories, offices, classrooms, land, facilities and the biological resources, such as plants, animals, etc.—must be adequately available to do the job. However, they will be of little value unless the human resources are adequate both as to numbers and quality. It is the human resources that I wish to discuss here.

Since man has directed our attention to outer space by sending satellites into the heavens, the people, as never before, have turned their attention to science, education, and technology. The two worlds are in a deadly race to achieve superiority in scientific competence. People are looking to science and technology in a large measure to guide the destinies of mankind. In the United States, great emphasis is being given to stepping up our educational program at the precollege and college levels, both in quantity and quality. While much of the cause of this activity is pointed to the physical and engineering sciences, all branches of learning are involved and all will be affected. This is having an effect in the sciences having to do with agriculture and the dairy industry. How well will agriculture and the dairy industry fare in obtaining the knowledgeable human resources to serve its needs? Competition is keen for scientific talents, and agricultural interests must see to it that their needs are met.

While we may expect that agricultural production, as in the past, will require fewer and fewer people as time passes, this is not the case with the research and educational people needed to serve agriculture through research, extension, and teaching. The task ahead in producing new information and getting it into proper use will require additional manpower, especially scientific manpower.

The job begins with attracting promising young people to take up careers in agriculture and in the dairy industry. While this begins at the primary level, the real effort must be made at the college training level. Agriculture and the dairy industry require many different kinds of talents, but here I am primarily interested in talent for research and education. Curriculums must be designed not only to attract and hold the interest of students but they must also be planned to prepare candidates adequately for careers in research, education, and extension. Likewise, the subjects must be taught interestingly, effectively, and efficiently. Curriculums must be flexible enough to prepare students to go in the direction of their interests; for example, more basic science courses for those interested in research.

College teachers must continually concern themselves about what they teach as well as how and why they teach it. In terms of current knowledge and needs, are the courses offered the most important? Are we teaching the right things? Are we putting the emphasis in the right places and on the most important exercises, to prepare students for advanced study or for careers in extension work? Are we as teachers well enough informed and effective enough in our teaching to encourage and stimulate the students under our charge in such a way as to most effectively prepare them for the future? With the graduate student, are we giving the proper guidance, leadership, and opportunity to develop individual competence and the creative thinking necessary for him to become an outstanding research worker? Are we requiring him to do enough independent and basic research along with his graduate study so that when his degree is in hand he is ready to take up a productive career in research? As teachers, we should be asking ourselves these and many other questions in relation to our own task, not only of attracting and preparing students for careers in agriculture and dairying but also in utilizing most effectively the human and physical resources available to us. In all of this work the college teacher must have not only the support of the agricultural industry which he serves but of the public. These industries also need to do more in making their requirements for talent known. At the same time, the potential rewards for work in these industries and in the research and educational areas serving these industries must be commensurate with those of competing vocations.

The extension worker, like the college professor, is a teacher. He, for the most part, is a teacher in adult education. In addition to a thorough and up-to-date knowledge of the subject matter, he must be knowledgeable in education techniques—the know-how of putting across ideas and information. The modern extension teacher is much less a jack-of-all-trades than his predecessor. He is a specialist. He has to be to be successful. He is working with a much different clientele. The modern farmer is more a business man and a specialist. This man wants to know how and why, and the extension man must be able to tell him and to show him that it will pay.

Our agricultural and dairy extension services have done outstanding jobs. The future is much more challenging than was the past. Are we prepared for the challenges ahead? Are the extension teachers keeping up with the revolutionary changes taking place on the farm, in processing and in marketing? Are we keeping up with new research findings? Are we using the most modern adult education techniques? Are we teaching the right things? Are we placing the greatest emphasis on the right programs to be most effective in serving the farmers interested in these specialized subjects?

Recognizing that the financial resources for extension work are limited, available manpower must be used as effectively as possible. Those of us in agricultural and dairy extension work

have a heavy duty before us to search out those things coming from the research laboratories and from elsewhere that are really important to the economic welfare of the farmer, to include in our individual extension programs.

Those of us in agricultural and dairy research have the responsibility to see that science in all of its broad aspects is applied, to continue to develop the information to meet the increasing needs of agriculture and the food and fiber industries. Agricultural science is very broad and includes biological, physical, and the engineering disciplines. It involves basic and applied science. Historically, the approach in agricultural and dairy research has emphasized work aimed at finding practical answers to pressing, practical, immediate questions. We have called on and utilized basic research as and where we found it or have depended on trial-and-error techniques to gain an answer. Until recently, only limited emphasis has been given to basic research. As a consequence, the backlog of basic information has dwindled to a low level. Recognizing this, and the dire need for more basic research to backstop the total research program required to support future agricultural needs, research leaders have been directing more of the available funds and other resources into basic research. Over the past 10 to 12 yr., the Agricultural Research Service, USDA, has increased the proportion of basic studies in its total research program from 7 to 22%. The state experiment stations have done equally as well. We feel that a larger share should go into basic research in future years.

This implies a shift in the research program and this is true. Those of us engaged in the direction of research have the obligation to continually review the research effort and to adjust it in terms of current needs and in terms of problems. In doing this, we must be conscious of the fact that moving scientific frontiers forward requires the accumulation of information on basic unknown scientific laws and principles. The new break-through of knowledge that will advance our objectives of more efficient agriculture will come, by and large, from basic research studies of unresolved biological and physical laws.

At the same time, we must not relent on the applied research programs that have contributed so much to the advancement in agriculture in the past, a kind of research that has also drawn the attention of scientists and agriculturists from all over the world. We must constantly bring into play a balanced research program in the aggregate in the U.S. This balanced program will emphasize a greater effort in basic research and a constant coordination to avoid unnecessary duplication of research activity. It will undergo constant review and adjustment, so that the total resources, limited as they are, will be directed to the most important problems and the elimination of the least necessary things. We must use our limited resources wisely, and intensify our efforts to develop and interpret research results quickly and effectively to the public.

To answer the questions that face agriculture today, and in the future, we must call on many diverse and specialized disciplines. The job requires the very best that is in us. This means that we must assemble scientists into research teams and groups, or in some cases as individual workers, best able to bring their talents to bear on the problem at hand, whether this be basic or applied research, or more likely a combination of both. It also means that we must be sure that there is being trained a sufficient number of new scientists to augment and replace the present force as needed.

Successful research depends largely on the ability of individual scientists. It also depends on an atmosphere in which able scientists can use their imaginations to generate new ideas and to put them to the test. While coordination, integration, and supervision of research are necessary, a large measure of self-direction is essential for productive work by scientists. Opportunity for individual initiative, opportunity to suggest new research problems, freedom of expression, and recognition must be supplied. Individual effort and the team approach to the solution of problems can be effectively combined in a good research environment. The team research approach can achieve coordinated effort and desired results and at the same time effect economy in costs. This approach is traditional in the cooperative research program of both state and federal agencies. At the same time, there is need for, and opportunity for, independent individual research activity, particularly in basic pioneering research problems.

It is traditional in the United States for scientists and educators to organize themselves into professional scientific societies. Among other things, these societies serve professional advancement, provide a medium for exchange of ideas, and a medium, on an over-all basis, to advance the cause in which they are engaged. The professional society also provides a forum for the member to present the results of his work at the annual meetings and a medium by which to publish reports of his research and educational accomplishments.

The American Dairy Science Association is the professional society in the dairy industry. All of you here, I am sure, are proud to be members of this Society. It is one of the large professional societies, made up of some 2,600 members and more than 600 student affiliate members. Its objectives are to advance, through science and education, the dairy industry. It has been eminently successful in this objective. The Association holds an enviable position in the dairy industry and among other professional societies.

The Association publishes the JOURNAL OF

DAIRY SCIENCE, thus offering a medium through which the great majority of the research results in dairy production and technology are published and made available to the public. Some 4,900 copies of this publication are issued each month and copies go to some 40 countries throughout the world. This Journal has been recognized as one of the top-flight technical journals in the scientific field.

The American Dairy Science Association has achieved its present high standing because of the caliber of its membership and because of the activity of the membership in the Association. Some 300 members are actively engaged on organized committee work of one kind or another. These men and many others give freely of their time to advance the cause of the Association, because they know that as the objectives of the Association are advanced, their own professional position is advanced.

One thing that all of us can do is to see to it that individuals interested and engaged in dairy sciences, especially the young people entering the field, become members of the American Dairy Science Association. Another important thing that we older members can do in this Association is to encourage the younger members and the student affiliates to become active in Association affairs and to promote their professional advancement. We all are engaged in a dynamic and important activity, and it is for us in large measure to determine whether the future will find us still the dairy industry leaders of the world.

In closing, I quote the inscription on an agricultural research mural recently dedicated in the Wilson Memorial Arch in the United States Department of Agriculture:

"Research has made American agriculture the most efficient and productive in the world. Scientific discoveries have been translated by farmers and agricultural industries into new practices, new products, and new distribution systems that have revolutionized our standard of living and our way of life.

"New knowledge and new machines have multiplied man's productivity manyfold. Manpower thus released from farming has built our industrial economy.

"Yet opportunities for further progress have never been greater. Each discovery helps to insure a more productive agriculture and a better life for all.

"Agriculture advances with research."

AMERICAN DAIRY SCIENCE ASSOCIATION AND INDUSTRY RELATIONSHIPS

H. F. JUDKINS, Secretary-Treasurer
White Plains, New York

The prevailing opinion among the membership of the American Dairy Science Association is that the Association should render all possible service to industry and, in turn, get more recognition from industry. This service could consist of:

1. Educating young men and women more thoroughly for careers in the various phases of the dairy industry.

2. Rendering assistance, especially in the processing areas, by making college graduates happier in industry. This can be done with material such as the new Dairy Industry Plant Training Manual and assisting industry to conduct training programs.

3. Developing a spirit of cooperation between industry and the universities, whereby industry will be of assistance in encouraging the best high-school students to study Dairy Science and assisting with scholarships. The large number of scholarships now being offered is greatly appreciated.

4. More understanding and cooperation between the universities and industry in the curriculum being offered. There appears to be some lack of understanding in industry about existing curricula and the type of educated men the universities are trying to train. Doubtless, these curricula can be improved. On the other hand, it seems that industry leaders do not understand just where and how the university graduate can serve best in the various business enterprises. Capable young men are in too great demand by competing industries to be satisfied with the dairy industry unless they are given opportunities that make it possible for them to attain remunerative and responsible positions in a reasonable time. University professors must understand that opportunities develop only through promotions, retirement, or death. This should be explained to students. In the normal course of events, the graduate entering the dairy industry must have a tremendous amount of patience in his journey to the top.

5. Continued expansion of research activities of members in the Association.

6. Publication of research in the JOURNAL OF DAIRY SCIENCE in a form that will be most helpful to other research workers.

7. Publishing research results in a way that they can be understood by the potential users of such research and by doing everything possible to encourage the use of practical research results throughout the industry. This is being done through THE A.D.S.A. REVIEW, published quarterly, which will supply the dairy industry with popular abstracts of research which has been published in the JOURNAL. University Extension workers also can be very influential in this movement.

8. Arranging programs for the Annual Meeting that will be attractive to our industry members. The program of the Dairy Manufacturing Extension group, held at the Annual Meeting at Utah State University last year, was a step in this direction.

ABSTRACTS OF PAPERS PRESENTED AT THE ANNUAL SOUTHERN DIVISIONAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

Jackson, Mississippi

February 6 to 8, 1961

Effect of feeding sorghum silage on the lipolytic activity in milk. P. E. JOHNSON AND R. L. VON GUNTEN, Oklahoma State University, Stillwater.

The influence of sorghum silage on the lipolytic activity in milk was studied by using a switch-back design. Two groups of four Holstein cows were used. All cows received the same ration, except that the alfalfa hay intake was increased to replace the sorghum silage in the ration of each group when the silage was removed. Samples were collected daily from the evening milking. Each sample was cooled immediately in ice water. Fat acid degree values were determined on the fresh samples and again after 48 hr. of storage at 32° F. Each time the groups were switched, the average fat acid degree value for the cows not receiving silage dropped below the average fat acid degree of the group receiving silage.

Daily variations in lipolytic activity in individual cow's milk. P. E. JOHNSON AND R. L. VON GUNTEN, Oklahoma State University, Stillwater.

Daily samples were collected from the evening milking of 12 Holstein cows over a 100-day period. The samples were divided immediately into three portions. Fat acid degrees were determined on one portion within 1 hr. after milking. The remaining two portions were immediately cooled to 32 and 42° F. and stored at these temperatures for 48 hr. before fat acid degrees were determined. Some day-by-day variations occurred in all portions of each individual cow sample, with the greatest variation in the portions held at 32° F. The variations in the lipolytic activity in the milk of some individual cows were much greater than they were in others. One cow's milk developed a rancid flavor 37 times during the 100-day period, whereas three other cows' milk never developed this flavor during the period.

Effect of feed and nutritional level of the cow on the development of rancidity in milk. R. Y. CANON AND G. H. ROLLINS, Agricultural Experiment Station, Auburn, Alabama.

Three groups of three cows each were assigned randomly to rations as follows: (1) hay, silage, grazing, full concentrate; (2) hay, silage, full concentrate; and (3) poor-quality hay, limited concentrate. Two trials were conducted: Trial 1 (2/26-4/11/60) with limited grazing, and Trial 2 (5/3-7/26/60) with full grazing on Ration 1. Samples were taken weekly from a complete P.M. machine-milking of each cow. Spontaneous and agitation-induced lipolysis were measured by acid degree value (ADV) determination after 24-hr. incubation at 4° C. In addition, temperature-induced lipolysis was determined in Trial 2.

During Trial 1, ADV's from spontaneous and agitation-induced lipolysis were higher ($P < .005$) on milk from cows on Ration 3 than Rations 1 and 2. No significant differences in ADV's as affected by rations were found in Trial 2. Correlations among ADV's resulting from spontaneous, agitation-induced, and temperature-induced lipolysis in Trial 2 were all positive ($P < 0.01$).

Microbiology of Cottage cheese made from frozen and daily transfer cultures. J. T. CARDWELL, T. J. OUSLEY, AND J. H. MARTIN, Mississippi State University, State College.

The effect of frozen and daily transfer cultures on the total bacteria, psychrophilic, coliform, and yeast and mold counts of Cottage Cheese was studied. Standard methods were followed.

To date, the data indicate that Cottage

Cheese made from starter using the frozen culture technique would have equal if not better keeping quality than that made from starter using the daily transfer system.

The microflora of Cottage cheese made from frozen and daily transfer cultures

Culture	Standard plate*	Psychrophilic*	Coliform**	Yeast and mold**
Frozen	68	78	78	56
Daily	63	69	69	44

* Values given are the per cent of counts which were less than 30,000 per gram of cheese.

** Values given are the per cent of counts which were less than ten per gram of cheese.

The results reported herein are based on the assumption that any contamination after the cheese was made was randomly distributed between the frozen and daily transfer batches. Difficulty was experienced in obtaining consistent counts on the starters.

Coliform bacteria in Cottage cheese. W. W. Overcast, J. D. Skean, and J. V. Britton, Jr., Agricultural Experiment Station, University of Tennesse, Knoxville.

A survey of Cottage cheese purchased at retail outlets showed a wide variation in coliform counts. These counts varied from less than one to 715,000 per gram on the day of purchase, with a median of 422. These counts were much too high and this research was planned to determine the extent of coliform contamination that could be expected in Cottage cheese manufactured under accepted procedures and plant practices. Sixty-four lots of cheese were manufactured from fresh skim or fresh skim fortified with nonfat dry milk solids. The skimmilk was pasteurized by the holding method at 144° F. for 30 min. The short set method was used for all lots and both medium- and large-sized curd was made. In the 64 lots, only three samples had an initial coliform count exceeding ten per gram; whereas, only two samples exceeded ten per gram after 11 days of storage at 41° F.

Persistence of contamination in lactic cultures. H. C. Olson, Oklahoma State University, Stillwater.

Regular lactic starter cultures were inoculated with a pure culture of some of the common organisms found in various dairy products. These included *Bacillus subtilis, Lactobacillus bulgaricus, Streptococcus durans, Streptococcus liquefaciens, Aerobacter aerogenes, Escherichia coli, Torulopsis sphaerica, Candida pseudotropicalis, Geotrichum candidum, Pseudomonas fragi,* and *Pseudomonas viscosa.* Counts were run initially and after each successive transfer and incubation at 72° F. for 14-16 hr. Differential counts were obtained by variations in medium used and incubation temperatures, such as to inhibit growth of the common lactic culture organisms.

Initial counts of the contaminants generally ranged from about one million to over 100 million. All of the organisms tested eventually disappeared from the lactic cultures on successive transfers, with no apparent impairment of the cultures. The coliforms and *S. liquefaciens* required from seven to nine transfers to reduce their numbers to less than one per milliliter, whereas the Pseudomonas species and the genera Bacillus and Lactobacillus were generally eliminated after four transfers.

The results suggest that foreign organisms present in lactic cultures due to contamination or to survival of the heat treatment of the milk are not likely to persist in a culture or to impair the quality of the culture.

Coliform counts as an index of the sanitary quality of raw milk. R. W. Henningson, Clemson College, Clemson, South Carolina.

Total and coliform counts were made at weekly intervals for approximately 80 milk producers over a 4-yr. period. Total and coliform counts were plated at one to one thousand and zero dilutions, respectively. TNTC notations were made when plates contained more than 500 colonies. A total count standard of less than 50,000 per milliliter was considered to be a reasonable index of the sanitary quality of raw milk.

The percentage of samples not meeting coliform standards of less than 100 per milliliter was four times as great as the percentage of samples not meeting a total count standard of less than 50,000 per milliliter. Only one-half of the samples not meeting total count standards failed to meet coliform standards. Only one-eighth of the samples not meeting coliform standards failed to meet total count standards. The average coliform count of all samples not meeting total count standards was well within the coliform limits. The average total count of all samples not meeting coliform standards was well within the total count limits.

Evaluation of the data indicate that there is little or no relationship between the total count and the coliform count. The data suggest that the use of coliform counts would be an entirely different method of evaluating the quality of raw milk.

Selective media for detecting spoilage organisms in dairy products. H. C. Olson, Oklahoma State University, Stillwater.

Standard plate count agar with 1 p.p.m. crystal violet added was used as the plating medium to detect spoilage organisms in milk and Cottage cheese. The use of 1 p.p.m. of brilliant green or 0.5 p.p.m. crystal violet plus

0.5 p.p.m. brilliant green was also effective, but the crystal violet medium was selected as the best.

Line counts on milk with the crystal violet medium showed a general correlation with the shelf life, in that high counts on the selective medium for a sample were generally followed by short shelf life at 45° F. Laboratory pasteurized samples generally showed less than ten organisms per milliliter on the crystal violet agar, with 18 out of 26 samples having counts of one or less per milliliter. The shelf life of the laboratory-pasteurized samples stored at 45° F. ranged from 16 to 38 days, whereas that of the same milk processed in the plant was usually less than ten days. The counts on the crystal violet agar for the plant-processed samples were higher in every instance than were the counts on the laboratory-pasteurized samples. Coliform counts did not indicate the degree of contamination as effectively as did the counts on crystal violet agar.

The shelf life of Cottage cheese could generally be predicted from the crystal violet counts on the fresh cheese and on the same cheese after holding 24 hr. at 70° F.

A comparison of the Kjeldahl, steam distillation, dye-binding, and formol titration methods for determining the protein content of milk. C. VANDERZANT AND A. H. MIAH, Texas Agricultural Experiment Station, College Station.

A comparison of the Kjeldahl protein (per cent protein) and formol titration values (milliliter 0.1*N* NaOH) indicated a mean conversion factor of 1.93. The mean protein values as determined by the four methods were very similar. The highest correlation was found between Kjeldahl and Buffalo Black protein values, the lowest between Kjeldahl and formol protein values. The correlation of 0.98 between Kjeldahl and Buffalo Black protein values was the same as those reported by others for Kjeldahl and Orange G protein values. The differences between individual steam distillation and Kjeldahl protein values were not greater than 0.1% in 80% of the samples. The comparative figures for Buffalo Black versus Kjeldahl, and formol titration versus Kjeldahl protein values were 75 and 65%, respectively. In 12% of the samples the differences between individual Kjeldahl and formol titration protein values were greater than 0.2%. A comparison of the individual Kjeldahl versus steam-distillation, and Kjeldahl versus Buffalo Black protein values, revealed only one sample with a difference of 0.2% protein between methods. A comparison of the duplicate readings (protein values) of the samples by either the steam distillation or Buffalo Black method showed differences between duplicates of not greater than 0.05% protein in over 90% of the samples.

The total protein, casein, and fat content of colostrum milk. O. T. STALLCUP AND F. E. REED, Arkansas Agricultural Experiment Station, Fayetteville.

Colostrum samples were obtained from five cows each of the Brown Syiss and Guernsey breeds and ten cows each of the Jersey and Holstein breeds. The first milking was obtained 8 to 12 hr. after calving and after the calf had nursed. Subsequent milkings were taken at approximate 12-hr. intervals until ten milkings were obtained. The average milk fat percentages for the first ten milkings were 5.0, 5.3, 5.6, and 5.7 for the Brown Swiss, Guernsey, Holstein, and Jersey breeds, respectively. They were very erratic and followed no particular trend in successive milkings. The average percentage of casein in ten milkings for all cows was 2.7 and was high in the first milking and declined with succeeding milkings. The average protein percentage for all breeds for the first ten milkings was 5.5 and ranged from 10.8 in the first milking to 3.9 for the tenth milking. The protein to fat ratio averaged 1.11 for all cows for the ten milkings. This ratio was 2.83 for the first milking and declined to 0.74 by the tenth milking.

Variations in the lactose and sodium chloride contents and the freezing point of certain reconstituted nonfat dry milks. E. W. CUSTER AND J. T. CARDWELL, Mississippi State University, State College.

A study was made of certain physical characteristics of nonfat dry milk solids from northern and southern sections of the United States. Nonfat dry milk solids were obtained from three southern and three northern powdering plants. The results showed that northern powder reconstituted to the same percentage total solids as southern powder resulted in a product having a lower freezing point, higher percentage of lactose, and higher percentage of NaCl. A 1-yr.'s study by seasons showed that northern powders reconstituted to 8.5% total solids had an average freezing point of −0.5096° C., while southern powders reconstituted to the same total solids froze at −0.4860° C. Northern powders reconstituted to 9.5% total solids had an average freezing point of −0.5666; southern powders at the same percentage total solids froze at −0.5391. Northern powders reconstituted from 8.5 to 9.5% total solids in increments of 0.2% showed the following freezing points: −0.5096, −0.5199, −0.5304, −0.5417, −0.5549, and −0.5666; whereas, southern powders reconstituted to the same per cent total solids froze at −0.4860, −0.4965, −0.5063, −0.5172, −0.5276, and −0.5391, respectively.

Northern powders contained an average of 51.88% lactose and 1.62% NaCl, while southern powders averaged 49.81% lactose and 1.42% NaCl.

Relationship of surface tension to variations in composition and temperature of fluid milk products. M. L. PEEPLES AND H. URBAN, Texas Technological College, Lubbock.

Regular skimmilk containing 0.3% fat and 8.7% MSNF decreased in surface tension from 49 dynes at 60° F. to 38 dynes at 170° F. Three reconstituted skimmilks containing 9, 12, and 20% MSNF showed surface tensions of about 49 dynes at 60° F. At 170° F., the values for these three products decreased to 40, 41, and 42 dynes, respectively.

The surface tension values of five nonhomogenized products (which contained 4, 10, 25, 30, and 40% fat, respectively) showed values of 48.5 dynes at 60° F. Sharp decreases in surface tension were noted between 60 and 90° F. The values for the five products at 90° F. were 38, 33, 32.5, 32, and 31 dynes, respectively.

Between 90 and 100° F., abrupt increases in surface tensions were noted. The values for the five fat-containing products ranged from 41 dynes for the 4% milk to 32 dynes for the 40% cream at 100° F. Between 100 and 170° F. the surface tension values decreased only slightly. The values ranged from 38 dynes for the 4% milk to 35 dynes for the 40% cream at 170° F.

Homogenized milk had higher surface tension values than nonhomogenized milk at all temperatures studied. Also, it is significant that homogenized milk failed to show an abrupt increase in surface tension between 90 and 100° F. The surface tension value for both products at 50° F. was 49 dynes; at 90° F., the value for homogenized milk was 44, and at 160° F. the surface tension was 42.5. The values of nonhomogenized milk were 38 dynes at 90° F., 41 at 100° F., and 39 at 160° F.

Variations in cultures of Geotrichum isolated from cream. E. R. GARRISON AND MARY ANNE HOLDAR, University of Arkansas, Fayetteville.

Three hundred cultures that resembled *Geotrichum candidum* were isolated from sour cream, purified, and studied. The following carbon compounds were assimilated by all cultures: dextrose, galactose, sorbose, D-xylose, ethyl alcohol, glycerol, mannitol, sorbitol, inositol, pyruvic acid, lactic acid, and succinic acid. All cultures grew without added vitamins, fermented dextrose and galactose, hydrolyzed milk fat, and liquefied gelatin.

The following carbon compounds were not assimilated by any culture: maltose, sucrose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, starch, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine hydrochlohide, potassium sodium saccharate, i-erythritol, dulcitol, alpha-methyl-D-glucoside, salicin, calcium-2-keto gluconate, and potassium-5-keto gluconate. All cultures failed to (1) grow at moderate osmotic pressure, (2) synthesize starch from carbohydrates, (3) ferment lactose, maltose, sucrose, inulin, raffinose, (4) utilize KNO_3.

The cultures varied in their ability to assimilate adonitol, citric acid, ethyl acetoacetate, and potassium D-gluconate. Variation in action on litmus milk was observed. All cultures grew at 4.4 and 36.1° C., 90 grew at 37° C., none grew at 37.5° C. On potato dextrose agar, 35 selected cultures gave the largest giant colonies at 26.6° (6) or at 29.4° C. (29); optimum pH ranged from five to eight, but growth was affected only slightly by pH within this range. All cultures survived heating in milk at 53.06° C. for 30 min., seven survived 57.2° C., none survived 58.6° C.

Rehydration of nonfat dry milk solids with home water supplies. A. V. MOORE, Texas Agricultural Experiment Station, College Station.

Distilled water and nine potable home waters were used to rehydrate one commercial lot of nonfat dry milk solids. The freshly rehydrated milks and those stored at 40° F. for 15 days had good flavor if the waters or the milks were pasteurized; otherwise, fruity, bitter, medicinal, astringent, and musty flavors occurred in some stored samples.

The curd tension of the raw rehydrated samples varied from 36 to 54; this appeared to be a function of the CO_2 and HCO_3 content. Pasteurization lowered the curd tension; the addition of $CaCl_2$ to the pasteurized milks raised it to as high as 74.

Normal acid development by a lactic starter was achieved in 14 to 15 hr., usually, but in waters high in bicarbonates there was a buffering effect for the first 5 hr. of incubation. Satisfactory starter aroma and acidity were developed better in high bicarbonate samples by doubling the usual amount of starter inoculum.

Phosphatase tests on butter. T. R. FREEMAN AND J. L. BUCY, Kentucky Agricultural Experiment Station, Lexington.

Reports from the butter industry indicated that certain procurement agencies of the federal government were occasionally rejecting shipments of butter because of positive phosphatase tests. The creameries maintained that the shipments of butter had been churned from properly pasteurized cream.

Objectives of the present study were (1) To ascertain the extent to which this could be a problem for the butter industry in Kentucky, and (2) if it was a problem of commercial significance, to devise methods for solving it.

Samples of freshly churned butter were transported to the Kentucky Agricultural Experiment Station laboratory and tested by the official phosphatase test (A.O.A.C., Method II). Six creameries using the batch method and two using the continuous method of churn-

ing furnished 347 samples, which were analyzed 24 hr. and 1, 2, and 4 wk. after churning. Twenty-eight per cent of the continuous, and 4% of the batch samples of butter were phosphatase-positive at the 24-hr. test period. Of 28 samples of continuous butter tested within 1 hr. or less of churning, 25% were negative at this period but positive at the 24-hr. period, and would be designated as false-positives. Tests for 24-hr. false-positives have not been made on batch-churned butter.

Effect of chocolate milk powder mixtures on the accuracy of the Pennsylvania TeSa, Gerber, and Babcock-Gerber tests for fat in homogenized chocolate milk. J. T. CARDWELL, Mississippi State University, College Station.

Results from 16 commercial chocolate mixtures indicate that the chocolate mixture used in preparing chocolate milk had a more pronounced effect on the per cent fat obtained by the Pennsylvania method than on results obtained by either the TeSa, Gerber, or Babcock-Gerber methods. The Babcock-Gerber method had the least variance in the deviations in fat percentage of chocolate milk from the fat percentage of the raw milk. These variances were 1.194, 0.072, 0.035, and 0.019% fat for the Pennsylvania, TeSa, Gerber, and Babcock-Gerber methods, respectively. The tests for the original milk averaged 3.57% fat; whereas, the chocolate milks averaged 2.52, 3.44, 3.48, and 3.40% fat as determined by the Pennsylvania, TeSa, Gerber, and Babcock-Gerber methods, respectively. The interaction existing between the effect of homogenization and chocolate mixture on the fat percentage values obtained by the Pennsylvania method was not evaluated.

The influence of nitrogen fertilization and harvest time on the nutritive value of pelleted coastal Bermudagrass hay. R. R. HARRIS, W. B. ANTHONY, J. G. STARLING, AND C. A. BROGDEN, Auburn University, Auburn, Alabama.

Nitrogen in amounts of 0, 160, and 320 lb. per acre was applied in split applications to well-established coastal swards. The forage was cut for hay three times during the season. After being field-cured, the hay was pelleted. Six uniform Hereford steer calves were used as test animals during conventional nutrient balance trials. Intake studies were also conducted with the same animals placed in individual stalls and fed the pellets to appetite. The average daily consumption of 9 lb. D.M. from October harvested hay supplied less digestible Calories than are required to maintain a 450-lb. liveweight calf. The average D.M. consumption of pelleted coastal hay cut during June or July was 11 lb. The influence of nitrogen on the nutritive value of coastal Bermudagrass hay was examined for the hay harvested July 28. Nitrogen application to the sward prior to harvest for hay had no significant effect ($P > .05$) on apparent digestibilities of dry matter, crude protein, cellulose, crude fat, or energy. The results indicate that pelleted coastal Bermudagrass hay is completely inadequate as the only source of nutrient for growing cattle. Deficiencies shown to exist for young cattle fed a diet of pelleted coastal Bermuda hay were: (1) insufficient intake of feed existed, even though the product was pelleted, (2) protein, (3) minerals.

An unbreakable esophageal-fistula cannula for collecting forage samples by grazing cows. L. L. RUSOFF AND L. E. FOOTE, Louisiana Agricultural Experiment Station, Baton Rouge.

Several types and sizes of plastic esophageal fistula cannulas were inserted successfully in dairy cows. When animals were allowed to graze on pasture, the cannulas cracked or broke, due to striking fences or trees. A thin stainless-steel cannula was designed which has proved to be useful in collecting samples of forage grazed. This cannula consists of 1.5 in. o.d. by .010-in. seamless wall tubing of stainless steel, type 347, and 4.25 in. long. The interior flange is 4.0 in. long and consists of 1.5 in. o.d. by .120-in seamless wall tubing of stainless steel, type 304. The surgical procedure as reported by Cook *et al.* (J. Animal Sci., 17:189. 1958) was followed for the introduction of the esophageal-fistula cannula, with modifications.

Changes in digestibility of cool-season forages during the spring grazing season. G. E. HAWKINS AND G. H. ROLLINS, Alabama Agricultural Experiment Station, Auburn.

Digestibility of oat and wheat forage dry matter was determined during the second week of March and the third week of April, with four cows per species grazing individual paddocks. Also, digestibility of dry matter of fescue forage was determined during the fourth week of March and the third week of April.

Mean digestibility of dry matter of the forages wes 72.8 and 65.5%, respectively, for the first and second trials. This 7.3 decrease ($P \leq 0.01$) was significantly correlated ($r = -0.479$) with the increase in crude fiber content of the forages. Also, digestibility of forage dry matter was correlated significantly ($r = 0.370$) with persistency of FCM production. Other significant correlations existed between digestibility of forage dry matter and intake of forage dry matter per 100 lb. of body weight ($r = 0.910$) and between the per cent crude fiber in the forage and the intake of forage dry matter per 100 lb. of body weight ($r = -0.793$).

The decline in digestibility of forage dry matter by species was oats 1.3%, wheat 2.2%, and fescue 1.5%. Forage dry matter intakes declined as digestibility decreased. Thus, the

milk production decline was due to decreased digestibility and decreased intake of the forages.

Relative feeding value of Coastal Bermuda grass silage and corn silage for lactating dairy cows. J. C. JOHNSON, JR., F. E. KNOX, AND B. L. SOUTHWELL, Georgia Coastal Plain Experiment Station, Tifton.

A relative evaluation of corn silage and Coastal Bermuda (CB) grass silage as the sole roughages was obtained from two double-reversal feeding trials and digestion trials using the lignin ratio technique.

In Trial 1, daily dry matter consumption per hundredweight was 1.73 lb. with corn silage and 1.71 lb. with CB silage. Feeding corn silage resulted in a significant increase of 1.54 lb. (6.0%) in average actual daily milk production per cow over that obtained with CB silage. Though not significant, there was a pronounced tendency for cows fed CB silage to gain more in body weight than cows fed corn silage (1.9 vs. −0.1 lb. per cow per day).

In Trial 2, daily dry matter consumption per hundredweight was 14.5% greater with corn silage (2.05 vs. 1.79 lb.). Feeding corn silage as compared to CB silage resulted in a highly significant increase of 3.80 lb. (14.1%) in average actual daily milk production per cow and a highly significant increase in average daily weight gains (0.73 vs. −0.61 lb.).

On a dry matter basis, the TDN content of corn silages and CB silages, respectively, was 55.3 and 46.9% for Trial 1 and 57.3 and 44.7% for Trial 2. Relative differences in estimated TDN content of the silages conform rather well with differences observed in the feeding trials.

Small-sample in vivo cellulose digestion technique for forage evaluation. J. W. LUSK, C. B. BROWNING, AND J. T. MILES, Mississippi Agricultural Experiment Station, State College.

The apparent digestibility of cellulose of several forages was determinel by conventional digestion trials and by 3-g. samples of forage placed in nylon sacks and suspended in the rumen of cattle.

Cellulose digestion of alfalfa was 56.5% by conventional methods, whereas 55.2, 54.9, 55.2, and 56.3% were obtained for 36-, 48-, 60-, and 72-hr. digestion periods, respectively, with small samples. Cellulose of Coastal Bermuda hay was 63.7% digested in a conventional trial, whereas 51.6, 55.4, 60.1, and 61.0% were digested by the small-sample technique in the respective hours stated. There was no significant difference between the digestion coefficients obtained with alfalfa or between the 72-hr. and conventional digestion coefficients for Coastal Bermuda.

Correlation coefficients of 0.66* and 0.80** were obtained in two trials when comparing simultaneous cellulose digestion by the two methods.

The digestion coefficients of eight forages determined by the small-sample technique, and the same forages in unrelated digestion trials, were significantly correlated (0.72*).

* Significant at 5% level.
**Significant at 1% level.

Two levels of distiller's grains and ground snap corn as preservatives for wheat silage. M. E. MCCULLOUGH AND L. R. SISK, Georgia Experiment Station, Experiment, Georgia.

Wheat, cut at the prebloom stage, was ensiled in 20-ton upright silos. The four silo treatments were: (a) 100 lb. corn, (b) 100 lb. distiller's grains, (c) 200 lb. corn, and (d) 200 lb. distiller's grains per ton of forage ensiled. The resulting silages were fed free choice to dairy cows. Grain was fed as the only supplement to the silage ration. Silages (a) and (b) were fed to two groups of four cows, with initial production of 26 lb. FCM. The cows consumed an average of 25.7 and 25.4 lb. dry matter of silages (a) and (b), respectively, or 2.85 and 2.78 lb. silage dry matter per 100 lb. body weight. They produced 22.7 and 23.8 lb. of 4% FCM milk. The difference was significant at the .05% level of probability. The two groups fed silages (c) and (d) had an average initial production of 36 lb. FCM. During the experiment, the cows consumed 25.4 and 25.7 lb. silage dry matter per day or 2.78 and 2.85 lb. silage dry matter per 100 lb. body weight on silages (a) and (b). Average FCM milk production was 29.5 and 32.5 lb. ($P = > .05$). The cows had a total dry matter consumption of 3.4 lb. per 100 lb. body weight on the silages with 200 lb. preservative, and 2.9 lb. when 100 lb. preservative per ton was used. The lower intake on the 100-lb. preservative level probably reflects the lower level of milk production of the cows fed the silage.

Performance of lactating dairy cattle fed millet silages harvested at two stages of maturity with and without corn as a preservative. E. B. MORGAN AND H. DEWITT ELLZEY, Southeast Louisiana Dairy and Pasture Experiment Station, Louisiana State University, Franklinton.

The treatments used in this investigation were: 1. Immature millet alone; 2. immature millet plus 150 lb. of ground snapped corn per ton of green forage as ensiled; 3. mature millet alone; 4. mature millet plus 150 lb. ground snapped corn per ton green forage as ensiled; 5. corn silage with no additive.

The immature stage of millet as used here was the stage at which time seed heads had not emerged, but the plants were flagging. Mature millet as defined in these trials was

the stage of maturity in which the seed heads were fully emerged and in the soft dough stage.

Intakes per animal on a dry matter basis, and expressing the D.M. as pounds consumed per 100 lb. of body weight were: 1.87; 2.03; 1.74; 1.85; and 1.93 for Treatments 1 through 5, respectively. It is noted that the immature millets, particularly Treatment 2, were consumed in greater quantities than the more mature stages of millet and corn silage. The highest dry matter intake was obtained from immature millet plus corn, with the lowest being corn silage alone.

Beginning levels of production for all cows was 34.0 lb. of FCM. The calculated grand mean for all treatments was 26.2 lb. of FCM for the study. Calculated treatment means were: 1—25.7; 2—28.7; 3—24.5; 4—27.0; 5—24.2 lb. of FCM. The highest level of production was provided by the immature millet plus corn, while the lowest was corn silage alone. Body weights were maintained for all cows, regardless of treatment.

Observations on the digestibility of sweet sorghum silage ensiled at four stages of maturity. D. S. RAMSEY, J. W. LUSK, AND J. T. MILES, Mississippi Agricultural Experiment Station, State College.

Although sweet sorghum is widely adaptable and highly productive, silage made from this crop has not been consumed or digested as well as several other silages tested. The effect of stage of maturity of Tracy sorghum on consumption and digestibility was studied, using two 4 × 4 Latin-square design digestion trials involving eight cows per trial. Sorghum was ensiled when the heads were in the following stages: fully flowered, milk, soft dough, and ripe (2 wk. after soft dough). All silages were fed ad lib. as the sole roughage. Two pounds of soybean oil meal were fed daily per cow in the second trial. For the respective stages, consumption of dry matter per 100 lb. of body weight was: 1958—0.84, 0.89, 0.89, 0.83; 1959—1.13, 1.25, 1.35, 1.31 lb.; digestibility of dry matter was: 1958—49.7, 52.1, 49.0, 45.8; 1959—51.5, 53.9, 54.0, 56.4%; digestibility of protein was: 1958—25.1, 25.5, 15.5, 26.5; 1959—41.8, 41.7, 32.1, 25.6%; and silo dry matter losses due to fermentation (by buried sack) were 1958—30.6, 29.7, 24.1, 30.0; 1959—34.4, 32.5, 25.8, 31.0%. Data on digestibility of cellulose and energy will be presented.

Nutrient composition and digestibility of corn silage as affected by fertilizing rate and plant spacing. R. A. ALEXANDER, G. A. BARDEN, J. F. HENTGES, JR., J. T. MCCALL, AND W. K. ROBERTSON, Florida Agricultural Experiment Station, Gainesville.

Corn was grown on Blichton fine sand and harvested in the early dent stage. Complete 4-12-12 fertilizer rates (lb/acre), nitrogen rates (lb/acre), and plant spacings (in.) were, respectively: Lot I, 400, 40, and 20; Lot II, 800, 80, and 20; Lot III, 400, 40, and 10; Lot IV, 800, 80, and 10. Digestibility data were collected on four steers and four wethers in two total collection digestion trials of 4 × 4 Latin-square design. Yields of air-dry forage (lb/acre) were: 2,680, 3,810, 4,220, and 5,360 for Lots I, II, III, and IV, respectively. At both the low and high rates of fertilization, protein content decreased with increasing plant population from 20- to 10-in. row spacing. Within each plant population, increase in fertilizer rate increased protein content but had little effect on other nutrient components. In Treatment III (high stand–low fertilizer), protein digestibility was significantly lower ($P < 0.01$) than in the other treatments. Digestibility of protein, energy, and ether extract was significantly increased ($P < 0.05$) in Treatments II and IV (high fertilizer). Increasing the plant population by closer spacings increased forage yields but decreased nutrient content (per cent) and digestibility. High fertilization (II, IV) at both low and high population increased total nutrients 1,106, 1,107 lb/acre and digestible nutrients 895, 751 lb/acre. High population (III, IV) at both low and high fertilizer rates increased total nutrients 1,509, 1,511 lb/acre and digestible nutrients 1,034, 890 lb/acre, respectively. All treatments of silage contained less than 0.015% potassium nitrate.

Lignin and some other constituents in Sudan grass and millet varieties. A. S. ACHACOSO, L. L. RUSOFF, C. L. MONDART, JR., AND F. L. BONNER, Louisiana Agricultural Experiment Station, Baton Rouge.

The lignin content and some other constituents of three varieties each of Sudan and Millet at different stages of growth were determined. The forages received 120 lb. of nitrogen per acre in two applications, and were cut at ten-day intervals. First cuttings on all varieties were made when plants reached 30-35 in. in height, and cut back to a 6-in. stubble.

Results on the effect of maturity on the chemical constituents showed that the lignin content of each variety increased significantly ($P < .01$) as maturity progressed. The average values ranged from 4.28 to 8.82% for Sudangrass and from 4.75 to 6.81% for Millet on the first and fifth cuttings, respectively. As was expected, crude protein decreased significantly ($P < .01$) from 19.8 to 8.6% for Sudangrass and from 25.3 to 7.3% for Millet, with sharp declines during the first two cuttings. Crude fiber increased significantly ($P < .01$) with maturity; however, no significant differences were found between values obtained after the second cutting for both species. Progressive increases in dry matter content

for all varieties tested were obtained at each successive stage of cutting. Nitrogen-free extract tended to be variable at the first three cuttings, after which the values increased up to the last cutting. Ether extract and ash also decreased with maturity.

Factors concerned in hot weather effects on growth and feed efficiency of dairy heifers. J. E. JOHNSTON, G. A. HINDERY, D. H. HILL, AND A. J. GUIDRY, Louisiana State University, Baton Rouge.

Nine yearling heifers including three Jerseys, three Holsteins, and three F_1 Red Sindhi–Holsteins were maintained in the Climatic Control Chamber for a 138-day period, starting November 3, 1959. An initial 88-day cool (60-70° F.) period was followed by a 40-day hot (75-95° F.) period. Average daily gain declined in all animals during the first 20-day exposure to hot conditions, but recovered during the second 20 days. These changes in gain were correlated with similar changes in dry matter intake. Daily water intake rose an average 17% on exposure to hot conditions. The greatest initial effects of hot conditions on rate of gain were observed in two of the three Holsteins and least in two of the three crossbreds. The estimated metabolic heat production showed a rise after exposure to hot conditions, as did PBI levels; however, thyroxine utilization rate declined. Differences in observed metabolic heat production and thyroid function do not appear to explain adequately differences in response to hot conditions, as measured by rates of gain and feed consumption.

Frequency of grain feeding by mechanical means and growth rate response of dairy heifers. O. H. HORTON AND G. S. NELSON, University of Arkansas, Fayetteville.

In this experiment a time-controlled automatic feeding device, designed by the junior author, was used to frequency-feed grain to dairy heifers over a 90-day period. A horizontal auger was used to deliver grain either into a holding box or into a manger. The grain falling into the manger was consumed, whereas the grain in the holding boxes accumulated until they were tripped by the timer. Feeding frequencies of two, four, and eight times a day were used. The total amount of grain fed per day was the same for all heifers. An average of 12.5 oz. of grain was augered at each interval when a group of three Holstein heifers for each frequency was being fed. In a second trial, using nine (three per lot) Jersey heifers, 9 oz. of grain were fed per feeding.

The response in body weight gains was similar to that reported previously by other workers. The mean body weight gains for heifers fed four and eight times daily were significantly greater than those fed two times daily. No differences were found in the mean body weight gains for heifers fed four and eight times a day.

Mechanical feeding reduced labor. The cost for electricity was approximately 0.019¢ per animal per day.

The importance of trace minerals in calf starter and milk replacer. E. W. SWANSON AND L. M. CARPENTER, University of Tennessee, Knoxville.

The effect of adding Fe, Cu, Co, Mn, Zn, and I to the ration was determined with 96 Jersey and 48 Holstein calves. They were divided into four equalized groups at birth: I—no mineral supplement; II—minerals added to milk; III—minerals added to starter; IV—minerals added to both milk and starter. Reconstituted skimmilk was fed from seven to 42 days to Holsteins and seven to 56 days to Jerseys. Calf starter was fed ad lib. up to 4 lb. per calf daily. Mixed hay was fed ad lib. Differences in growth among the groups were nonsignificant. For the first 7 wk. the calves fed supplemented skimmilk averaged 57.0 lb. gain compared to 58.7 lb. for the controls. The 1- to 16-wk. gains averaged 122, 120, 125, and 121 lb., respectively, for groups I to IV. There were no significant differences among groups in feed consumption. Differences in hemoglobin concentrations due to mineral supplementation of milk or starter were not statistically significant. Determinations were also made of hematocrit, blood Ca, P, and PBI. None of the measures of evaluation used indicated any benefits from trace mineral supplementation.

Comparison of different calf starters for early weaned calves. L. J. BUSH, G. V. ODELL, AND M. G. YANG, Oklahoma State University, Stillwater.

Thirty-two male Holstein calves were used in an experiment to compare four different calf starters. A 2×2 factorial arrangement of treatments was used, with the addition of a commercial enzyme preparation (Agrozyme) to the starter as one variable and steaming of the grains in the mixture as the other variable. Thus, the four starter groups were: (1) steamed grains plus added enzymes; (2) steamed grains without enzymes; (3) unsteamed grains plus added enzymes; and (4) unsteamed grains without enzymes. The calves were started on experiment when four days of age and were fed whole milk at the rate of 10% of initial body weight for 14 days and at the rate of 8% of initial weight for an additional ten days. Each calf starter was fed ad libitum, starting when the calves were four days of age and constituting the sole diet after the calves were weaned abruptly at 28 days of age. Up to 8 wk. of age, the average daily gain of the calves was 1.34, 1.22, 1.17, and 1.35 lb. for Groups 1, 2, 3, and 4, respec-

tively; at 16 wk. of age the average gain for the same respective groups was 1.67, 1.62, 1.48, and 1.68 lb/day. There were no significant differences among treatment groups with respect to weight gains, feed consumption, blood glucose levels, rumen pH, or general health of the calves in this experiment.

Gradual vs. abrupt weaning of dairy calves from a limited level of milk replacer. W. J. MILLER AND C. M. CLIFTON, University of Georgia, Athens.

Thirty-two Holstein and 16 Jersey calves were fed milk replacer as the only source of milk products from five days of age until weaned. A conventional system of gradually increasing the amount, followed by a gradual reduction, was compared with a system in which a calf was given the same quantity each day until weaning. By this abrupt system, the Holsteins and Jersey calves were given 9 and 7 lb. of milk replacer per day for 33 and 37 days, respectively. Those on the conventional system received the same totals over a 45-day period. These treatments were combined in a factorial design in which four calf starters were used. There were no significant differences in weight gains, starter consumption, incidences of diarrhea, or days of diarrhea among the treatments. When diarrhea occurred, the only change made in feeding and caring for the calves was to give 2 oz. of Modified-Marcol B-75 twice per day. During the year this study was in progress, 31 instances of diarrhea were observed and treated with this product. Of these, 22 appeared normal after one day, six others after two days, and another one the third day. The remaining two were treated by personnel of the School of Veterinary Medicine.

Evaluation of the growth of dairy calves on a constant feeding regime in southern Louisiana. E. J. STONE, E. B. MORGAN, AND J. B. FRYE, JR., Louisiana Agricultural Experiment Station, Baton Rouge.

Four years' observations on growth and feed intake have been made on 119 Holstein and 79 Jersey female calves from birth through 12 wk. on a constant feeding regime. Mean birth weights for the Holsteins and Jerseys were 74.9 and 48.4 lb., respectively, while body weight at 12 wk. was 153.3 and 116.2 lb. Analysis of variance of body weight for the Holsteins gave highly significant differences ($P < .01$) in birth weights and body weight at 12 wk. among years and among sires in years; whereas, gain in body weight (0-12 wk.) was highly significant ($P < .01$) among years and significant ($P < .05$) among sires in years. For the Jersey breed, differences in birth weight among years and among sires in years were not singificant. However, body weight at 12 wk. was highly significant ($P < .01$) among years and significant ($P < .05$) among sires in years. Body weight gain (0-12 wk.) was significant at the 1% level among years and among sires in years.

When gain in body weight over the 12-wk. period was adjusted for differences in birth weight, highly significant differences among sires within years were obtained for the Holstein breed.

Best Fitting Curves for body weight and heart girth are presented for the Holstein and Jersey breeds. Comparisons with USDA and Ragsdale standards show no significant differences between the slopes of the curves for the Louisiana animals with USDA and Ragsdale standards.

Pasture vs. barn feeding for raising calves. B. F. HOLLON AND R. D. MOCHRIE, Coastal Plain Research Station, Willard, N. C.

Four female and eight male Jersey calves were raised on pasture from four through 112 days of age. The pasture consisted of new seedings of small grain–Crimson clover for the period October-April, Ladino clover–Fescue for April-May, and Starr millet for June-July. An equal number of calves was simultaneously raised in a barn and fed Coastal Bermuda grass hay ad libitum for roughage. All calves were supplemented with very limited amounts of whole milk to 35 days of age and with calf starter up to a maximum of 2.5 lb. per day to the end of the trial. Calves on pasture weighed 56 lb. initially; consumed 123 lb. of milk, 189 lb. of starter (seven of the 12 averaged 7.1 lb. of hay during inclement weather); gained 0.98 lb. per day liveweight, and increased in height at withers 5.90 in. Corresponding values for barn-raised calves were: 56, 119, 201, 156, and 0.83 lb. per day and 4.75 in. The greater weight gains and increases in height for calves on pasture were both statistically significant at $P < 0.05$. Observation of fecal samples and digestive tract contents indicated little difference between treatment groups in number or kinds of intestinal parasites.

Quality codes in evaluating feeds in EDPM-DHIA records. R. SAM JONES, JR., Agricultural Extension Service, Auburn, Alabama.

Feeding schedules developed in machine-processed DHIA records are the most accurate available to dairymen today—if forage data used in calculating these schedules are accurate. Three major errors occur in forage data reported for machine calculation, (1) erroneous quality codes, (2) erroneous dry matter content, (3) inaccurate daily intake.

In Alabama, a forage testing program has been developed to furnish more accurate data on forages. A trial program was set up in 1959 to evaluate the reliability of quality codes developed from chemical analysis of forages, in maintaining expected milk persistency when feeding schedules were devel-

oped on a basis of the chemical analysis of forages fed. Results of this study will be given.

A survey of quality code and dry matter content figures used by supervisors shows a wide variation from codes and dry matter content figures developed from chemical analysis of forages. This variation will be discussed.

Care and accuracy of testing equipment used by DHIA associations. R. R. RICH AND M. E. SENGER, North Carolina State College, Raleigh.

Fifty spring-balance scales were checked for accuracy at the 5-, 10-, and 25-lb. weights. Scales tended to become less accurate at the 25-lb. weight. Seven or fourteen per cent of the scales deviated 0.3 of a pound or more from the actual weight at this level. Forty per cent of the scales showed no deviation from the actual weight.

Sixty-eight milk-o-meters were checked. They varied in length of use from 1 to 52 mo. The length of use since any meter was last checked varied from 1 to 31 mo. The average use of the meters was 8.6 days per month. Fifteen, or twenty-two and eight tenths per cent, of the meters deviated from the check weight by 0.6 of a pound or more. There was a tendency for the meters to underweigh. Some inaccuracies were due to improper cleaning of the meter.

The speed of the centrifuge was checked for accuracy by the use of a tachometer. The speed ranged on 24-bottle machines from 700 to 950 revolutions per minute. Twenty-two of the 40 machines checked were below the recommended speed, 15 were above, and three were within the recommended range. Only a few of the machines were considered excessively slow or fast. There was practically no difference between the speed of an empty or loaded centrifuge.

Thermometers were checked for accurate readings. Seven of 34 thermometers checked were off five degrees or more when compared to a tested thermometer. There tended to be more inaccuracies in the higher temperature ranges.

Critical appraisal of the central processing program for DHIA records. W. S. GRIFFITH, Virginia Polytechnic Institute, Blacksburg.

The DHIA program has gained limited acceptance due to the inability of the Extension Service to communicate effectively with the industry. There are more dairymen who feel that they do not need records than there are who recognize this need. Relatively few extension workers or dairymen appreciate the National Cooperative Dairy Herd Improvement Program (NCDHIP). However, declining profit margins are causing dairymen to re-evaluate their need for records.

Although the present NCDHIP offers many advantages to dairymen today, additional research is needed to keep the program geared to the future needs of the industry. The present program assumes that forage consumption within a herd is directly proportional to and in the same ratio as body weight. It assumes the cow is equally efficient in utilizing the nutrients in a forage regardless of the quantity fed. It assumes that each pound of grain fed produces equal response in milk production, regardless of the level of feeding. The replacement value of energy in good hay is the basis of estimating all forage costs. Grain-feeding recommendations fail to consider the value of the milk produced.

There is a grave danger that our testing programs may be fitted to the electronic machinery rather than to the needs of the industry.

Experiences in mastitis control in Florida. C. B. PLUMMER, JR., Florida Extension Service, Gainesville.

It is estimated that mastitis is costing the dairy industry of Florida 5.5 million dollars annually. This is an average of $23.50 per cow per year.

Florida has had a state mastitis control program for 12 yr., and it has always been under the direction of the Florida Livestock Board.

When the dairymen request assistance, the supervisor visits the dairy. He first explains the program and stresses the feat that cooperation by the owner is necessary. If the owner is agreeable, the following steps are taken:

1. The plant and ground are carefully studied and necessary physical changes noted.
2. Milking equipment is examined. Equipment for checking the vacuum, pulsators, and electrical current has been added recently.
3. A stable test using the strip cup is made to determine infected animals.
4. Milking methods are observed.
5. A report listing infected animals and quarters is given to the owner.
6. Recommendations are made suggesting changes that should be made in the physical plant and milking habits.
7. If treatment is indicated, it is suggested that the owner seek professional advice for proper medications.
8. Follow-up visits are made by the supervisor.

One approach to a mastitis prevention and control program. S. L. KALISON, Virginia Polytechnic Institute, Blacksburg.

An effective mastitis prevention and control program must include four phases. These are good herd management, proper milking procedures, accurate and complete diagnosis, and treatment based on the diagnosis.

Application of herd management procedures and milking methods depends on the man milking the cows. The diagnostic and treatment phases require the services of the veterinarian and the diagnostic laboratory. A successful program depends on the joint effort and cooperation of the dairyman, veterinarian, and laboratory.

Failure of the dairyman to fulfill his role in the total program will nullify the results of treatment, even if it is effective, and will create more mastitis than can be successfully treated. It is self-evident, therefore, that the first step in any approach to the mastitis problem is an educational program that must:

1. Inform the dairyman of the extent of the losses due to the disease.
2. Make the dairyman aware of the fact that something can be done about it.
3. Present the information relative to milking methods and herd management essential to mastitis control.
4. Convince the dairyman to apply the recommended practices to the entire herd continuously.

The next phase of the progression is the initiation of the clinical aspects of a total prevention and control program, including diagnosis and treatment.

Responsibility of extension workers to artificial insemination programs. RICHARD E. BURLESON, Federal Extension Service, U. S. Department of Agriculture, Washington 25, D. C.

The purpose of the National Association of Artificial Breeders is to breed Better Cattle for Better Living. The guiding principle of the Cooperative Extension Service is helping people to help themselves. Extension's role in artificial insemination programs is in keeping with both of these themes.

Dairymen are interested primarily in breeding better cattle in order to improve their standard of living. Thus, Extension's responsibility falls into two categories. First, extension workers have the responsibility of encouraging the use of the most outstanding dairy sires available. In most cases, this will be through artificial insemination. Secondly, they have the responsibility of making information available which will insure maximum results from artificially sired offspring.

The use and value of outstanding herd sires can be emphasized through the DHIA-proved sire program, the various breed registry organization programs, local artificial breeding associations, and county or state breed organizations. Dairy extension activities should include a clarification of terminology, requirements for registration, breeding difficulties, and the need for good service by the artificial breeding association or semen-producing business.

After conception to semen from outstanding sires, Extension's major responsibility is to assist dairymen in developing a calf-raising and heifer development program which will realize the benefit of good breeding.

Responsibilities of bull studs to artificial insemination programs. E. W. NEASHAM, Louisiana State University, Baton Rouge.

The responsibility of the artificial insemination program would be to:

1. Provide bulls of outstanding genetic background that will improve type and production.
2. Provide semen from bulls that pass all N.A.A.B. health requirements.
3. Employ only well-trained technicians, who are energetic, reliable, and capable of getting cows settled.
4. Provide service in all of the operating territory.
5. Hold regular meetings with members.
6. Provide information through news letters or otherwise.
7. Provide pedigrees that are complete and up-to-date.
8. Have services available for planned matings.
9. Hold training schools that will provide for enough practice and technical background so as to assure a high degree of technicians' success.
10. Encourage technicians to provide information on the latest management practices.

Effects of nitrogen and other gases on livability and fertility of frozen bovine spermatozoa. J. D. ROUSSEL, T. E. PATRICK, AND H. C. KELLGREN, Louisiana State University, Baton Rouge.

This study was divided into two parts, with the first phase being preliminary. The split-ejaculate technique, using five mature dairy bulls, was employed to determine the effects of carbon dioxide, oxygen, and nitrogen gases on the freezability and livability of spermatozoa. Flushing ampules with nitrogen gas proved superior to the control and other gases and methods of application. Both oxygen and carbon dioxide were found to have deleterious effects on freezability and livability of semen.

The second phase of the experiment was expanded to include both laboratory and fertility observations. The split-ejaculate technique, using six mature bulls, was employed to study the effects of flushing ampules with nitrogen on freezability, livability, and fertility of spermatozoa. Nitrogen gas had no appreciable effect on freezability; however, motility of semen stored at various intervals after thawing (livability) was consistently higher for the treated samples than for the control. The differences were significant at the 0.01 level.

Fertility, based on 30- to 60-day nonreturns

to 277 first services, was 79.0% for the nitrogen-treated semen as compared to 76.7% for 292 services with nontreated semen. Breeding efficiencies based on 60- to 90-day nonreturns were 74.0 and 71.5%, respectively. These differences were not significant ($P > 0.05$).

Effects of feeding protamone to bulls on semen production, libido, and physiological responses. T. E. PATRICK, J. D. ROUSSEL, H. C. KELLGREN, AND J. O. SHELWICK, Louisiana State University, Baton Rouge.

Bulls in Group I served as a control and those in Group II were fed 1.25 g. of Protamone daily per 100 lb. of body weight. Protamone feeding was started November 16, 1959, and continued through March 15, 1960, with the level being gradually reduced during the last 15 days. October 26 through November 15, 1959, was used as a base period. Observations were continued for 30 days after Protamone feeding was completed, in order to determine the after-effects.

Group means for all observations were adjusted to correct for initial differences. Although the adjusted means for volume of semen were slightly higher for the treated group during all three periods, the differences were not significant. In contrast, the adjusted means for motility were slightly higher for the control animals. An analysis of variance revealed highly significant differences in concentration during all three periods, indicating that Protamone had a depressing effect on sperm production.

A sharp decline was observed in per cent motility and an increase in abnormal sperm during the reduced protamone feeding and after protamone was discontinued. There was no noticeable change in libido during these periods; however, semen quality gradually returned to normal within 45 days after Protamone feeding was discontinued. The feeding of Protamone to bulls failed to show any appreciable benefits in semen quality. However, there was a significant improvement in libido in the group of bulls receiving Protamone supplement. The feeding of Protomone to bulls did not increase body temperature or respiration rate.

Certain factors affecting the solids-not-fat and total solids in milk. O. T. FOSGATE, University of Georgia, Athens.

A total of 352 individual samples of A.M. and P.M. milk and 176 composite samples from Jersey and Holstein cows were tested for SNF. The percentages of SNF for A.M. milk were higher than the P.M. percentages for both breeds. Mean percentages for A.M. and P.M. SNF were 9.94, 9.69, and 8.92, 8.68 for the Jerseys and Holsteins, respectively. Within-cow coefficients of variation (C.V.) ranged from 0.4-8.6% for the Jerseys and from 0.4-8.4 for the Holsteins. Composite Jersey samples had a range of 9.58-10.0% SNF; composite Holstein samples had a range of 8.56-8.96% SNF. Coefficients of variation between cows were 2.2 and 2.3%.

Two hundred and forty samples, collected over a 5-wk. period, were tested to determine percentages of SNF and TS found in different portions of the milk. Samples were taken at the end of the first 30 sec., first 90 sec., and first 120 sec. of milking. The percentages of SNF in the Jersey milk were found to be the highest in the first portion and decreased as milking progressed. This progression was not definite in the Holsteins. Mean percentages SNF and TS at 30, 90, and 120 sec. for the Jerseys were 9.78, 13.16; 9.55, 15.57; and 9.44, 17.66. For the Holsteins, 8.73, 11.05; 8.85, 13.05; and 8.64, 14.24, respectively.

Influence of certain environmental factors on the fat and solids-not-fat production of Jersey cows. J. C. JOHNSON, JR. AND R. L. GIVENS, Georgia Coastal Plain Experiment Station, and USDA, ARS, AERD, Tifton.

Multiple regression analyses were used to examine the influence of daytime average dry and wet bulb temperature, solar radiation, hours above 80° F., maximum and minimum temperature, daytime average shaded and nonshaded black globe temperature, rainfall and wind speed on milk volume, and on fat and SNF per cent. Partial regression coefficients indicated that relatively few of the above climatic variables significantly affected fat and SNF per cent. For the most part, increases in the temperature variables resulted in significant declines in milk volume. Multiple correlation coefficients between all climatic variables and fat per cent, SNF per cent, and milk volume were highly significant and not different between shaded and nonshaded treatments, except for AM fat per cent. Of the variation observed in per cent AM fat, AM SNF, PM fat, PM SNF, daily average fat, and daily average SNF, only 5.4, 9.6, 6.0, 5.4, 6.5, and 9.0%, respectively, could be attributed to the combined influence of the ten climatic variables. Of the variation observed in AM milk volume, PM milk volume, daily 4% FCM volume, respective percentages of 32.8, 32.4, and 29.3 could be attributed to the combined influence of the ten climatic variables.

Genetic and environmental differences among dairy herds. N. R. THOMPSON, V. L. BALDWIN, AND G. C. GRAF, Virginia Agricultural Experiment Station, Blacksburg.

Lactation records for milk production of Jersey cows were analyzed statistically for herd and intra-herd components of variance. One lot of 4,408 records came from groups of half-sisters located in two or more herds. A second lot of 1,389 records came from groups of half-sisters located in single herds. The difference in relative size of the herd com-

ponents from the two groups was multiplied by four-thirds. The resulting value, when expressed as a fraction of the entire herd component, indicated that only 11% of the variance due to differences in milk yield could be attributed to heredity.

Effect of vitamin B_6 deficiency in the gonads of male rats. N. PATI, The Agricultural and Technical College of North Carolina, Greensboro.

Sixteen male albino rats of the Holtzman strain were randomly divided into four groups. Each of them was approximately 70 to 80 days of age and weighed 125-5 g. The three treated groups were fed the Pyridoxine-deficient diet and each of them was injected with 0.5 mg. percentage of Des-oxy-Pyridoxine hydrochloride subcutaneously until the animals showed the symptoms of vitamin B_6 deficiency (14 days). The fourth group was fed a control diet which contained 1.0 mg. Pyridoxine per 100 g. of Stock diet. Three weeks after the experimental animals showed the typical syndrome of B_6 deficiency, the treated as well as the control animals were killed and testes were studied histologically. The histological examination from cross section of testes from B_6-deficient rats revealed lack of interstitial tissue and atrophied condition of cells of Sartoli. Secondary spermatocytes did not complete maturation division. Small numbers of spermatozoa were observed in the lumen of seminiferous tubule, in comparison to the number of secondary spermatocytes and spermatids. The results indicated possible interference of vitamin B_6 with the effect of follicular-stimulating hormone on growth and development of seminiferous tubules, and spermatogenesis.

Livestock improvement in the Middle East. E. M. HARMON, Hendersonville, N. C.

Flocks roamed the arid lands of the Middle East long before the dawn of written history. Most every disease known to the livestock world is prevalent. Feed and water are very inadequate. High mortality of livestock and day-to-day demand for milk, meat, and eggs to prevent human starvation have, over the centuries, been such as to completely discourage selective breeding.

In Iran, the feed problem was tackled through (1) teaching the use of 650,000 tons of beet pulp formerly wasted because they thought it useless, (2) growing of alfalfa, (3) ensiling of sorghums and other roughages. Improved breeding of cattle is being accomplished with Brown Swiss bulls in 12 insemination centers, Sindhi bulls in one, and Water Buffalo in another. Crossbred cows are producing three times as much as natives and adding millions of dollars annually to Iranian income. Similar results are being attained with poultry, by distributing cockerels from over 200 New Hampshire flocks. Upgrading of native sheep, and breeding mules to replace oxen, are making enormous contributions. Of equal importance are 89 vaccination teams which police the country to stamp out disease outbreak. The outstanding significant point is that these activities are all being carried on by Iranian technicians being trained by Americans to carry out their own programs.

Call for Papers for the 1961 Meeting of the Eastern Section of the American Dairy Science Association

The Eastern Division of the American Dairy Science Association will meet on August 7 and 8 at the University of Massachusetts, Amherst. This is the second time that the annual meeting will be held at a land-grant institution. Last year the University of New Hampshire was the host institution. Titles of papers to be presented at the meeting must be sent to Secretary W. F. Shipe, Department of Dairy and Food Science, Cornell University, Ithaca, New York, by June 1. A more detailed announcement will be mailed to the Eastern Division members.

All abstracts must be prepared according to the CALL FOR PAPERS FOR THE 1961 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION, which appeared in the JOURNAL OF DAIRY SCIENCE from September, 1960, through January, 1961, and which was prepared by G. W. Trimberger of Cornell University.

BOOK REVIEWS

THE ARTIFICIAL INSEMINATION OF FARM ANIMALS. Ed., Enos J. Perry. Rutgers University Press, New Brunswick, New Jersey. 3rd Rev. Ed. 430 pp. 1960. $6.50.

This book was first published in 1945, about 7 yr. after the advent of artificial insemination in the United States, and has been revised three times, 1947, 1952, and 1960. Since its original printing, the quantity of data concerning artificial insemination, accumulated through continued research, has increased manyfold. The third edition contains much new information (21 chapters written by 15 well-known authors) that reflects the latest experimental findings of researchers throughout the world and presents it in a well-organized and in an easily understood way.

Perhaps the main attribute of this book is that it is pointed toward the practical application of scientific findings and principles. Because of its practical approach, this book is excellent for use by livestock breeders, managers and technicians of artificial breeding organizations, veterinarians, students, and teachers. This should not detract from the fact that it is also a good book for graduate students and researchers as a starting place for a review of literature in this field. Particularly helpful, in this respect, is the fact that specific references are found throughout each chapter and a complete list of references is printed at the end of each chapter. This allows the reader to gain appreciation for eminent workers in the field and the various contributions made by each.

The third edition has been changed considerably. The chapter concerning the advantages and limitations of artificial insemination has been eliminated, as these are obvious to anyone familiar with the field. A new chapter on the evaluation of semen by chemical analysis by Dr. T. Mann is a welcomed and worth-while addition. This chapter contains a list of the chemical components of semen from several species, which is an important addition if one is to research factors involved in the bio-chemical processes of sperm and thus to improve storage and processing techniques. A new chapter on the artificial insemination of buffaloes also lends itself to a better understanding of the world-wide use of artificial insemination. An extremely worth-while addition is the chapter on frozen semen by H. A. Herman. This chapter presents a brief history of frozen semen, discusses the advantages and disadvantages of it, and adequately presents details on the freezing technique and related factors. Another area of extreme importance is that of disease and artificial insemination. This chapter has been completely revised by two new authors: D. E. Bartlett and L. L. Larson. Six other chapters concerning the artificial insemination of swine, poultry, dogs, and horses and jackstock, and chapters on shipping semen, and the role of hormones in reproduction, have been vastly revised. These chapters reflect the enormous strides made in advancing the artificial insemination of other species and of new knowledge gained in related areas. In addition, the other chapters not mentioned here have been revised sufficiently so that they contain up-to-date information.

It is the opinion of the reviewer that this third edition has been greatly improved and that anyone associated with artificial insemination would do well to obtain a copy.

F. D. BARTLETT, JR.
University of Illinois
Urbana, Illinois

VETERINARY MEDICINE, by D. C. Blood and J. A. Henderson. 1,008 pages. The Williams and Wilkins Company, Baltimore, Maryland. 1960. Price, $15.

This book has been written for veterinary medical students, practicing veterinarians, and other animal disease workers. The scope has been restricted mainly to diseases of cattle, horses, hogs, and sheep. Exotic diseases found only in limited areas are not considered in this book. The book is divided into two sections. The first part deals with the general principles of diseases as they affect individual organs or systems. Specific disease entities are considered in the second part. The layout makes the book an easily used reference when information on an individual case is sought. A standard list of headings has been adhered to in the discussion of each disease.

A list of references is included for each disease entity, but the discussions are not a complete review of the literature. The references appear to have been included so that the reader may refer to them for further detail.

Historical material and illustrations have not been included and details of bacteriology, pathology, and parasitology have been kept to a minimum. Etiology, transmission, pathogenesis, clinical signs, clinical pathology, necropsy findings, diagnosis, treatment, and prevention are the major headings for each disease.

The special medicine section is divided, except for the discussion on mastitis, primarily on the basis of causative agents. Diseases caused by bacteria, viruses, rickettsia, fungi, and protozoa are given detailed consideration, as are those due to deficiency of specific nutrients. The section on toxicities caused by chemical and plant poisons is brief but adequate. Diseases caused by allergic reaction and by heritable factors are included. The manner in which the book is indexed makes it an easily used reference.

ROGER P. LINK
College of Veterinary Medicine
University of Illinois, Urbana

when only the finest
will do – **NESTLÉ'S**

Join the Dairy Processors
who know – there is no
finer quality than NESTLÉ'S
Cocoas, Granules and Liquors.

PETER'S® • RUNKEL'S® **NESTLÉ'S®**

THE NESTLÉ COMPANY, INC.
WHITE PLAINS, N. Y.

®Trade Mark Reg.

DIFCO

Media for

MYCOLOGY

DIAGNOSTIC

. . . for the isolation, identification and cultivation of pathogenic fungi. These media are also widely used in phytopathological studies. Several are neutral in reaction, giving optimum conditions for growth of a variety of fungi. The following may be prepared as selective media by the adjustment of reaction, addition of antibiotics or other agents:

Bacto-Brain Heart Infusion Agar
Bacto-Sabouraud Dextrose Agar
Bacto-Sabouraud Maltose Agar
Bacto-Littman Oxgall Agar
Bacto-Mycological Agar
Bacto-Mycological Broth
Bacto-Corn Meal Agar
Bacto-Corn Meal Agar with Dextrose
Bacto-Bean Pod Agar
Bacto-Prune Agar
Bacto-Lima Bean Agar

CONTROL

. . . for sanitary and sterility procedures as well as for general use in mycological procedures:

Bacto-Sabouraud Maltrose Broth
Bacto-Sabouraud Liquid Medium
Bacto-Malt Extract
Bacto-Malt Agar
Bacto-Neurospora Culture Agar
Bacto-Potato Dextrose Agar
Bacto-Mildew Test Medium
Bacto-W.L. Nutrient Medium
Bacto-W.L. Differential Medium

CLASSIFICATION

. . . and nutritional studies of fungi:

Bacto-Yeast Morphology Agar
Bacto-Yeast Carbon Base
Bacto-Yeast Nitrogen Base
Bacto-Czapek Dox Broth
Bacto-Czapek Solution Agar
Bacto-Vitamin Free Yeast Base

THE DIFCO MANUAL, *NINTH EDITION*,
including descriptions of these media and their use, is available on request.

DIFCO LABORATORIES

DETROIT 1, MICHIGAN